KINETIC ANALYSIS OF SELF-REPLICATING PEPTIDES: POSSIBILITY OF CHIRAL AMPLIFICATION IN OPEN SYSTEMS

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(Received 11 December 2002; accepted in revised form 21 March 2003)

Abstract. A simplified kinetic model scheme is presented that addresses the main reactions of two recently reported peptide self-replicators. Experimentally observed differences in the autocatalytic efficiency between these two systems – caused by variations in the peptide sequences – and the possible effect of chiral amplification under heterochiral reaction conditions were evaluated. Our numerical simulations indicated that differences in the catalytic performance are exclusively due to pronounced variations in the rate parameters that control the reversible and hydrophobic interactions in the reaction system but neither to alterations in the underlying reaction network nor to changes in the stoichiometry of the involved aggregation processes. Model predictions further demonstrated the possible existence of chiral amplification if peptide self-replication is performed under heterochiral reaction conditions. Pointing into the direction of a possible cause for biomolecular homochirality, it was found that in open flow reactors, keeping the system under non-equilibrium conditions, a remarkable amplification of enantiomeric excess could be achieved. According to our modeling, this is due to a chiroselective autocatalytic effect and a meso-type separation process both of which are assumed to be intrinsic for the underlying dynamics of heterochiral peptide self-replication.

Keywords: amplification of enantiomeric excess, autocatalysis, biomolecular homochirality, chiroselective autocatalytic effect, kinetic analysis, peptide, self-replication, stereoselectivity

1. Introduction

The design and study of artificial self-replicating systems play a key role in obtaining closer insight into the early evolution of living systems and the dynamics of their possible building blocks (Orgel, 1992; Bag and Von Kiedrowski, 1996; Robertson *et al*., 2000; Cousins *et al*., 2000). Self-replication can be understood as a process of molecular cloning governed by autocatalytic kinetics and combined with highly specific molecular recognition. These processes can display effective information transfer, dynamic error correction (Severin *et al*., 1998) and – as recently shown by Saghatelian *et al*. (2001) – even the ability to give rise to chiral selectivity.

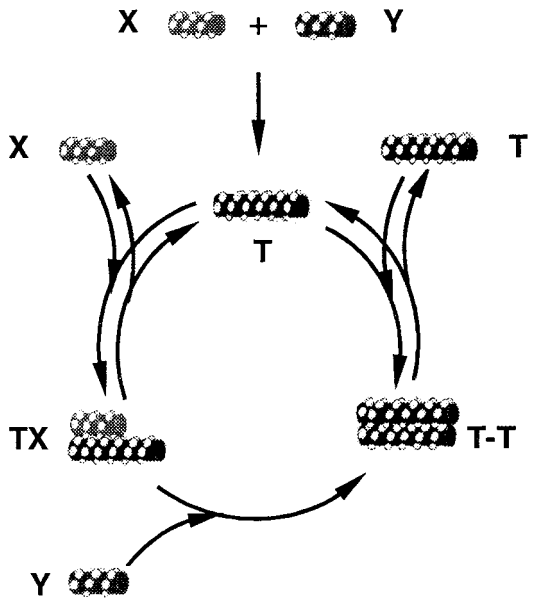
Origins of Life and Evolution of the Biosphere **34:** 497–512, 2004.

Prominent prototype non-enzymatic self-replicators were either based on, for example, short deoxyribonucleotide oligomers involving the template-directed binding of two trideoxyribonucleotides (Von Kiedrowski, 1986) or on purely synthetic molecules including an amino adenosine – pentafluorophenylester linkage (Tjivikua *et al*., 1990). More recently also peptide-driven systems have been reported (Lee *et al*., 1996; Yao *et al*., 1998) to show self-replicating behavior that was so far quite unexpected (Eigen, 1971). These systems may open the way to investigate self-replication 'on a basis for wider than Watson-Crick base-pairing in polynucleotides' (Kauffman, 1996) and, consequently, could provide an additional perspective to the 'RNA world' hypothesis (Joyce, 1989) that is restricted to self-replication in terms of carbohydrate but not protein-based chemistry.

Peptide self-replication, as for example demonstrated by Ghadiri and co-workers (Lee *et al*., 1996; Severin *et al*., 1997), can be observed in a remarkably simple set-up by using a 32-amino-acid α-helical peptide having the sequence found in the natural yeast transcription factor GCN-4. This 32-residue peptide can act as a template and catalyzes its own formation by accelerating the amide bond formation between a pre-activated and electrophilic 17-residue peptide fragment and a nucleophilic 15-residue peptide fragment that were composite to the template. In this process, the two peptide fragments and the template apparently form a ligation complex in form of a coiled-coil motif.

As it is a usual practice for analyzing self-replicating systems, autocatalytic kinetics have been experimentally confirmed by initially adding increasing amounts of the template product to the reaction mixture that clearly prompted in an acceleration of the observed initial rate. A similar approach was also followed by Issac and Chmielewski (2002) by using synthetically designed peptide sequences showing particularly high autocatalytic efficiencies. The basic design principle that is supposedly common for all peptide self-replicators is outlined in Scheme 1. It indicates the autocatalytic cycle as an effective machinery for the increasing formation of the template product during the course of the reaction.

Previously, we presented a kinetic analysis of the natural-based, i.e., homochiral, Ghadiri-type self-replicator (Rivera Islas *et al*., 2003a) as well as a modeling attempt (Rivera Islas *et al*., 2003b) for a heterochiral scenario in which peptide fragments composed of L- and D-amino acids were used and a chiroselective effect favoring the formation of the homochiral over the heterochiral template product has been experimentally observed (Saghatelian *et al*., 2001). This astonishing effect, generated by cross-catalytic interactions between the L- and D-peptide fragments and the respective template species, has been discussed as a possible model system for the origin of biomolecular homochirality. However, considering a closed system, such as originally used for the experimental studies, our simulations showed that chiral amplification effects are strongly limited. This is mainly because the reaction system is operating with an initially present chiral pool in which no additional generation of chiral matter takes place. In turn, effective chiral amplification – as has been proposed in terms of generalized kinetic models (Frank, 1953;



Scheme 1. Simplified representation of a self-replicating peptide system in terms of a template-directed, autocatalytic reaction network. X and Y refer to the two composite peptide fragments. If X stands for the electrophilic fragment E, then Y is the nucleophilic fragment N and *vice versa*. T = monomeric template product, T–T = template dimer, TX = either TE or TN intermediate association complex.

Kondepudi and Nelson, 1985) as well as observed in some experiments (Kondepudi *et al*., 1990; Soai *et al*., 1995; see also for reviews: Bonner, 1991; Kondepudi and Asakura, 2001 and references therein) – require not only a high degree of stereoselectivity but also the net generation of chiral species in order to feed the amplification process.

In this article, we extend our modeling to a more generalized description of the dynamic properties of peptide self-replicators and apply our approach to a system that has been recently reported by Issac and Chmielewski (2002). In respect to the chiroselective effect, we follow further the question if more efficient chiral amplification can be predicted for experiments under open flow conditions in which a continuous inflow of reactants and an outflow of reaction mixture are taken into account. Open flow conditions, maintaining a system far from equilibrium, can be regarded as viable for chirally autocatalytic reaction systems as well as to mimic prebiotically realistic environments.

TABLE I

List of processes, reactions and corresponding rate laws in the kinetic core model. E (electrophilic) and N (nucleophilic) stand for the two composite peptide fragments (if X = E then Y = N and conversely)

Process	Reaction	Rate law	Step
Direct formation of the template template product	E + N $\rightarrow$ T	$R_{\mathrm{I}} = k_1$ [E][N]	I
Reversible association equilibrium between template and fragment	T + X $\rightleftharpoons$ TX (X = E or X = N)	$R_{\mathrm{IIa}} = k_2$ [T][X]	IIa
		$R_{\mathrm{IIb}} = k_{-2}$ [TX]	IIb
Catalyzed formation of template dimer	TX + Y $\rightarrow$ T–T (X = E or X = N)	$R_{\mathrm{III}} = k_3$ [TX][Y]	III
Reversible association equilibrium between template and template	T + T $\rightleftharpoons$ T–T	$R_{\mathrm{IVa}} = k_4$ [T]^2	IVa
		$R_{\mathrm{IVb}} = k_{-4}$ [T–T]	IVb

2. Kinetic Modeling

2.1. Dynamic properties of the kinetic core model

The kinetic core model (Table I), designed for a homochiral scenario of peptide self-replication, has been already shown (Rivera Islas *et al.*, 2003a) to reproduce well the experimentally observed data from different experimental settings that were reported by Ghadiri and co-workers in a number of publications (Lee *et al.*, 1996; Severin *et al.*, 1997; Saghatelian *et al.*, 2001). Known variations in the experimental conditions in these reported settings have been made in respect to temperature or to the concentrations of auxiliary compounds. For all these cases, the model gave an excellent fitting to the observed kinetic curves. During our fitting attempts, we observed deviations in the adjusted rate parameters between the experimental series. These variations were most probably due to changes in the experimental conditions and, consequently, have been interpreted by the model as differences in the obtained rate parameters. Nevertheless, the excellent data fitting indicated that the model structure could serve as a basic description for the processing of peptide self-replicators in general.

However, as shown in Table II, variations in the amino acid sequence of the used reactants and consequently of the formed product species in the studied Ghadiri experiments were very small and obviously not affecting significantly the observed kinetics. In turn, major changes in the amino acid sequence, also shown in Table II, apparently can have dramatic effects on the kinetics by altering the catalytic activity of the template species. It appears instructive to test the core model against major changes in the amino acid sequence in order to reveal if the basic processing in these systems is the same as in the Ghadiri self-replicator and eventually to derive

TABLE II

Amino acid sequences of template products in some reported peptide self-replicators. The hyphen indicates the position where ligation has occurred

Sequence	Reference
RMKQLEEKVYELLSKVA-CLEYEVARLKKLVGE	Lee *et al*., 1996[a]
	Severin *et al*., 1997[a]
	Severin *et al*., 1998
RVKQLEKKVSELLKKVA-CLEXEVARLKKLVGE	Saghatelian *et al*., 2001[a]
KMAQLKKKVQALKSKVA-CLKXKVQALKKKVAQR	Kennan *et al*., 2001
KMAQLKKKVQALKSKVA-SLKXKVQALKKKVAQR	
LEKELYALEKELA-CLEKELYALEKEL	Issac and Chmielewski, 2002[b]

[a] Former data fitting of the Ghadiri-type self-replicator (Rivera Islas *et al*., 2003a).
[b] Present data fitting; X = lysine-ε-NHCO-Ar.

the possible dynamic origin for the differences in the catalytic efficiencies. We have chosen the experimental data reported by Isaac and Chmielewski (2002). These authors used a synthetically designed peptide system (sequence given in Table II) that reportedly performed a catalytic rate enhancement that was close to known enzymatic systems.

Autocatalytic efficiency in self-replicating systems is usually evaluated by analyzing the initial rate (ir) as a function of the added concentration of the template product ($[\mathrm{T}]_0$) at the beginning of the reaction (Von Kiedrowski, 1993),

$$ir = a[\mathrm{T}]_0^{\rho} + b\ , \tag{1}$$

where $a[\mathrm{T}]_0^{\rho}$ stands for the autocatalytic term in respect to the initial rate and b for the non-catalyzed direct formation of the template.

In the presence of product inhibition, which is usually caused by the dimerization of the template product, a parabolic growth in the initial rate approaching $\rho = 0.5$ can be expected indicating a drastic reduction of the autocatalytic effect. On the other hand, in absence of any product inhibition, an exponential growth ($\rho = 1$) should be observed as it is the case for prototype quadratic autocatalytic reaction systems. For the formerly studied experiments of Ghadiri and co-workers, a value of $\rho \approx 0.65$ was found while Issac and Chmielewski (2002) reported for their system a value of $\rho \approx 0.91$ that signifies nearly the absence of any product inhibition.

Figure 1 shows the fitting of the kinetic data from Issac and Chmielewski (2002) by our core model (Table I). It demonstrates that the simultaneous data reproduction addresses well the series of experiments, in which 4 different initial amounts of the template (0, 10, 20, and 40 μM) have been initially added. This indicates high coherence in the underlying dynamics between the Ghadiri- and the Chmielewski-

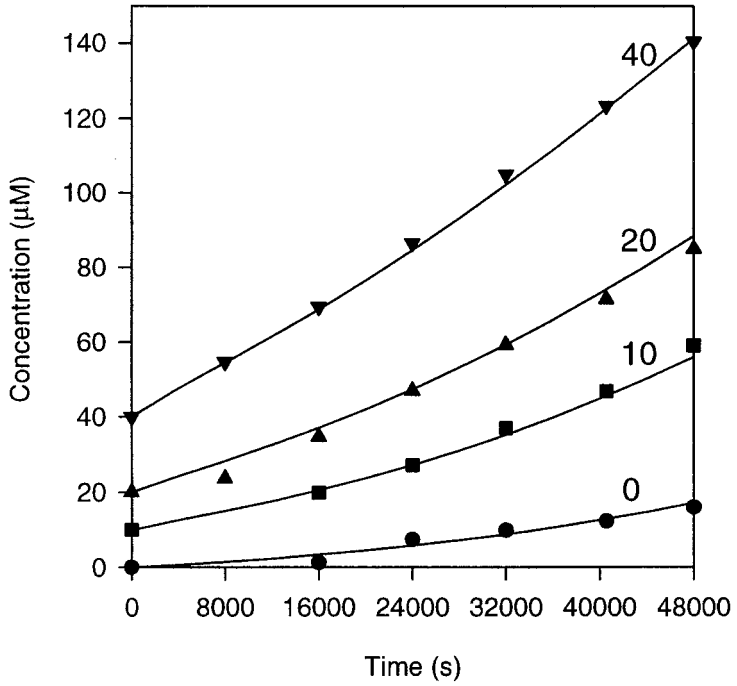


Figure 1. Product formation vs. time in the Chmielewski-type peptide self-replicator. Data points taken from Issac and Chmielewski (2002). The continuous lines show the data fitting by the kinetic core model (Table I). Varying initial concentrations of the template product are indicated by the respective figures (in μM) in the graph. Initial concentrations of the two peptide fragments 500 μM each, T = 23 °C.

type peptide self-replicator, gives rise to additional validation of our modeling approach, and allows a closer dynamic analysis of the later system by the model.

Table III summarizes the orders of magnitude of the rate parameters that were obtained by the data fitting of the Chmielewski-type reaction, system (B), in comparison to those that have been formerly evaluated (Rivera Islas *et al.*, 2003a) for the Ghadiri-type self-replicator, system (A).

Using an empirical modeling approach, Issac and Chmielewski (2002) already observed that the uncatalyzed background reaction in their system to form the template (step I) appears to proceed significantly slower than in the Ghadiri case, system (A). In contrast to that, as shown by our modeling results, the template-fragment associations (step IIa) at the beginning of the autocatalytic cycle seem to occur notably faster. The key difference between system (A) and (B) becomes obvious by comparing the association equilibrium constants K_{ass}(TX) and K_{ass}(TT). The ratio $K_{ass}(TX)/K_{ass}(TT) \approx 3 \times 10^{-8}$, system (A), and about 6×10^{-2}, system (B), reflects the higher catalytic efficiency of system (B) over system (A). It appears that in system (B) the product inhibition, expressed by the dimerization of T, is

TABLE III

Optimized rate parameters (*log*) and equilibrium constants (*log*) obtained from the kinetic core model as shown in Table I. (A) Ghadiri-type (average values from three independent experimental data fittings), and (B) Chmielewski-type peptide self-replicator

Steps	Process	Rate Constant	(A)	(B)
I	E + N → T	(k_1)	–1.4±0.7	–3.2
IIa	T + X → TX T + X → TX	(k_2)	1.5±0.2	2.6
IIb	TX → T + X	(k_{-2})	0.9±0.4	–0.7
III	TX + Y → T–T	(k_3)	4.4±1.3	–0.8
IVa	T + T → T–T	(k_4)	5.1±3	0.5
IVb	T–T → T + T	(k_{-4})	–3.0±1.3	–3.9
–	K_{ass}(TX)	(k_2/k_{-2})	0.6±0.6	3.3
–	K_{ass}(TT)	(k_4/k_{-4})	8.1±4.3	4.5

much weaker while the autocatalytically important association of the two peptide fragments to the template is largely stronger.

Issac and Chmielewski (2002) have assumed that their value of $\rho \approx 0.91$ implies the formation of tetrameric ligation complexes like, for example, 3T + X + Y ↔ T–T–T–X–Y. We have already stressed that these interpretations – considering only the very initial stage of the reaction and based on an empirical treatment – can be misleading (Rivera Islas *et al.*, 2003a). As indicated by our present modeling approach, in which exclusively dimerizations have been considered, it is not necessary to assume the formation of multimer complexes to obtain an acceptable fitting of the experimentally observed data. This is not to deny that multimers may exist in the mentioned system. We want to point out that considering multimers is not needed from the dynamical point of view and that an *a priori* assumption of their presence derived only from the value of exponent 'ρ' can be wrong.

2.2. CHIRAL AMPLIFICATION

Chiral selectivity in a prebiotically relevant self-replicating system has been first shown by Ghadiri and co-workers (Saghatelian *et al.*, 2001) by using peptide fragments composed of L- as well as of D-amino acids. In contrast to the naturally-based system, in which the homochiral template (TLL) was the exclusive monomeric product species, four different template species, two homochiral (TLL and TDD) and two heterochiral (TLD and TDL) are obtained.

TABLE IV

Processes considered for the kinetic model of the heterochiral Ghadiri-type system (see for details: Rivera Islas *et al*., 2003b). Note that the chiral combinatorics conditions apply only on the bimolecular processes. (i, j, k, m = D or L; if X = E then Y = N and conversely)

Type of reaction	Chiral combinatorics conditions	Number of processes
$E_i + N_j \rightarrow T_{ij}$	$\forall i, j \quad i = j$	2
$E_i + N_j \rightarrow T_{ij}$	$\forall i, j \quad i \neq j$	2
$T_{ij} + X_k \rightarrow T_{ij}X_k$	X = E and $i = k$ or X = N and $j = k$	8
$T_{ij} + X_k \rightarrow T_{ij}X_k$	X = E and $i \neq k$ or X = N and $j \neq k$	8
$T_{ij}X_k + Y_m \rightarrow T_{ij}T_{km}$	X = E and $j = m$ or X = N and $i = m$	16
$T_{ij}X_k + Y_m \rightarrow T_{ij}T_{km}$	X = E and $j \neq m$ or X = N and $j \neq m$	16
$T_{ij} + T_{km} \rightarrow T_{ij}T_{km}$	$i = k$ and $j = m$	4
$T_{ij} + T_{km} \rightarrow T_{ij}T_{km}$	$i = k$ and $j \neq m$ or $i \neq k$ and $j = m$	4
$T_{ij} + T_{km} \rightarrow T_{ij}T_{km}$	$i \neq k$ and $j \neq m$	2
$T_{ij}X_k \rightarrow T_{ij} + X_k$	None	8 + 8 = 16
$T_{ij}T_{km} \rightarrow T_{ij} + T_{km}$	None	4 + 4 + 2 = 10

A clear-cut effect of chiral selectivity, i.e., an increase in the diastereomeric excess (de),

$$de = \frac{(\mathrm{TLL} + \mathrm{TDD}) - (\mathrm{TLD} + \mathrm{TDL})}{\mathrm{TLL} + \mathrm{TDD} + \mathrm{TLD} + \mathrm{TDL}} \tag{2}$$

has been observed during the course of reaction. It corresponds to the predominant formation of the homochiral template species TLL and TDD.

In a previous contribution (Rivera Islas *et al*., 2003b), we have shown that the kinetic core model (Table I) – after adaptation to the heterochiral conditions – can reproduce satisfactorily well the experimentally observed time evolution of the homochiral and heterochiral template species as observed by Ghadiri and co-workers. For this adaptation, we used a combinatorial approach leading to a considerable growth in involved species from 6 (homochiral case) to 34 (heterochiral case) and a drastic increase in the kinetic steps from formerly 6 to 98 (see Table IV). Based on the successful data fitting, we concluded that the homochiral experiments can be understood as a special case of the more general heterochiral scenario.

A high degree of chiral selectivity as displayed by the Ghadiri-type heterochiral peptide self-replicator and the essential presence of an autocatalytic reaction network can be considered as a basic requirement to observe chiral amplification, i.e., an autocatalytically enhanced increase of the enantiomeric excess, ee, for instance in respect to the enantiomeric couple TLL/TDD during the course of reaction,

$$ee(\mathrm{T}) = \frac{\mathrm{TLL} - \mathrm{TDD}}{\mathrm{TLL} + \mathrm{TDD}} \tag{3}$$

Unlike the observed increase in *de*, an amplification of *ee* could be only monitored under non-racemic initial conditions. Although respective experimental results are still not available, the presence of this effect would be indeed of high impact in the pending discussion about the origin of biomolecular homochirality.

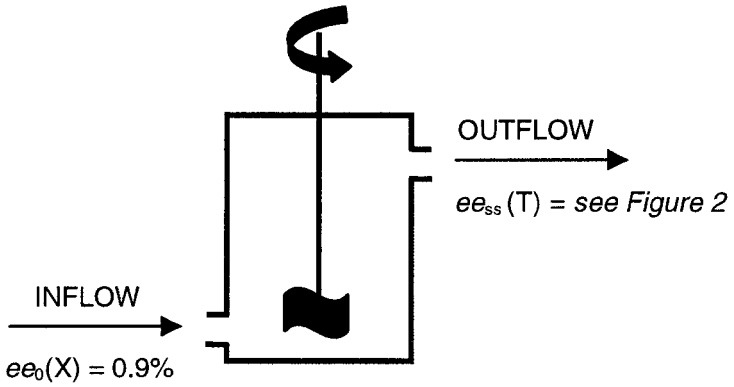
Based on our earlier model calculations (Rivera Islas *et al*., 2003b), we predicted the presence of a positive nonlinear stereochemical effect (Avalos *et al*., 1997; Girard and Kagan, 1998) in the heterochiral Ghadiri-type self-replicator in which the product enantiomeric excess exceeded slightly the enantiomeric excess of the initially present reactants. However, from the quantitative point of view, this effect remained small when compared to the possible theoretical outcome of already mentioned generalized kinetic models of stereospecific autocatalysis in which any small initial enantiomeric bias can be in principle amplified up to nearly $ee = 100\%$. Also experimental systems, such as pioneered by Soai *et al*. (1995), can show considerably higher pronounced amplification effects than we predicted for the peptide system. On the other hand, these kinds of systems, including air and moisture sensitive reactants, are definitely less related to an immediate prebiotic perspective.

One of the driving forces for the predicted amplification of *ee* in the Ghadiri-type system appears to be a weak chiroselective effect in which the consumption of one type of reactant enantiomer, for example EL and NL, is autocatalytically accelerated over the consumption of their optical antipodes like ED and ND. A second and more pronounced origin for the predicted amplification of *ee* is the formation of *meso* type species – such as the template dimer TLL–TDD – that are gathering chiral matter to an optically inactive product, which in turn leads to an increase in *ee* in respect to the remaining chiral species as it is typically observed in a racemate crystallization (Jacques *et al*., 1981). However, amplification of *ee* in this case goes to the cost of actually present chiral material that is directly proportional to the optical rotation.

The Ghadiri-type system shows a conceptual limit for the formation of a sufficiently high optical yield because during the course of the reaction process no chiral matter in addition to that of the reactant species is generated but only redistributed over the product species. Furthermore, a significant amount of chiral material is simply annihilated by the formation of *meso* species.

Also in an open flow reactor (Scheme 2) the above mentioned conceptual limit basically holds but the system can be constantly kept in non-equilibrium conditions and supplied with fresh reactant solution. Hence, if an infinite amount of chirally enriched reactant solution can flow into the reactor, it can lead to the formation of an infinite quantity of chirally enriched product species. The enantiomeric excess of the template product species, TLL and TDD, is then expressed by their stationary values $ee_{ss}(T)$.

Figure 2 shows the result of simulations of the Ghadiri-type heterochiral system under open flow conditions. The reactor was constantly supplied with a reactant mixture that had a slight enantiomeric imbalance in favor of the L-reactant species



Scheme 2. Diagram of a continuous-flow well-stirred tank reactor (CSTR) considered for the computer simulation of peptide self-replication in the heterochiral Ghadiri-type system (Saghatelian *et al.*, 2001). The net rate of inflow of each species is given by $R_f = k_f$ ([A]$_0$ – [A]), where k_f (s^{-1}) stands for the flow rate parameter, [A]$_0$ for the inflow concentration and [A] for the actual present concentration of species A in the reactor. For the simulations shown in Figure 2, an inflow of EL, NL, ED, ND was considered, where [EL]$_0$ = [NL]$_0$ = 50.0 μM and [ED]$_0$ = [ND]$_0$ = 49.108 μM corresponding to ee_0(X) = 0.9%.

NL and EL over ED and ND, ee_0(X) = 0.9%. Resulting values of ee_{ss}(T) were taken as a function of the adjustable flow rate parameter k_f.

It turns out that ee_{ss}(T) sensitively depends on the flow rate parameter k_f. For the considered inflow concentrations as shown in Figure 2, ee_{ss}(T) is going through a steep maximum at $k_f \approx 1.8 \times 10^{-4}$ s^{-1} and climbs to about 12.6% at this peak value. Further increasing k_f leads to a drop in ee_{ss}(T) that slowly is approaching zero at high values of k_f (for scaling reasons not shown in Figure 2). This corresponds to a scenario in which the reactor is being completely 'washed out', i.e., in which the extent of reaction also becomes zero. A similar picture is also obtained for the corresponding values of the stationary optical rotation. In contrast to that, at smaller k_f, the stationary enantiomeric excess approaches a value of 1.7% that is consistent with the equilibrium value found for a corresponding simulation under batch conditions, i.e. for a set-up as used for the Ghadiri-type experiments.

Hence, if choosing an optimal flow rate parameter, our modeling predicts significantly higher chiral amplification efficiency for the case the reaction is performed under flow conditions.

Figure 3 demonstrates the existence of a clear nonlinear relation between ee_{ss}(T) and the enantiomeric excess of the inflowing reactants, ee_0(X), at the optimum k_f shown on Figure 2. This nonlinearity arises from the already discussed basic dynamics of the heterochiral Ghadiri-type self-replicator, i.e. the presence of a chiroselective autocatalytic effect and the formation of *meso* products.

Under flow conditions, this nonlinear effect appears to be significantly stronger than under batch conditions. However, comparing the ee_{ss}(T) in the flow reactor with its transient value, $ee_{transient}$(T), in a batch reactor, it appears that they ex-

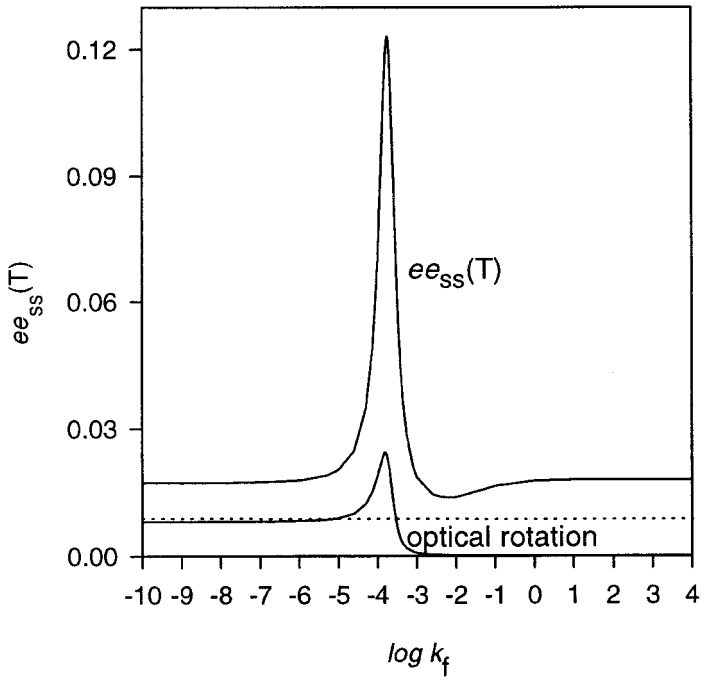


Figure 2. Simulation of the Ghadiri-type heterochiral peptide self-replicator (Saghatelian *et al.*, 2001) under open flow conditions. Stationary values of the product enantiomeric excess, ee_{ss}(T), (upper curve) and of the optical rotation (lower curve, in arbitrary units corresponding to [TLL] – [TDD]) are plotted as a function of the flow rate parameter k_f (*log* scale). The horizontal dotted line indicates the reactant enantiomeric excess, ee_0(X) = 0.9%.

actly match. This result is expected as flow conditions can only assist to 'freeze' the system at an optimum extent of reaction at which a maximum of the batch enantiomeric excess was observed.

Nevertheless, the consideration of a cascade scenario, i.e., the alignment of several flow reactors that are coupled in series so that the output of one reactor will be the input of the subsequent reactor, can – under specific conditions – lead to an astonishingly high ee_{ss}(T). A cascade approach in respect to RNA replication by serial transfer experiments has been already discussed in the past (Spiegelman, 1971; Eigen and Schuster, 1982), however, without referring to possible chiral implications.

First simulation attempts were made under the assumption of chirally enriched inflow of the reactant species EL, NL, ED, ND into the first reactor and then the repetitive transfer of all 34 involved species at their particular stationary concentrations and at the same k_f into the following reactor. This scenario, however, resulted in a continuous decrease in ee_{ss}(T) starting with the second reactor. This can be explained by a kind of 'wash-out' effect due to the high concentrations of the product species that have been formed in the first reactor. For instance, a significant

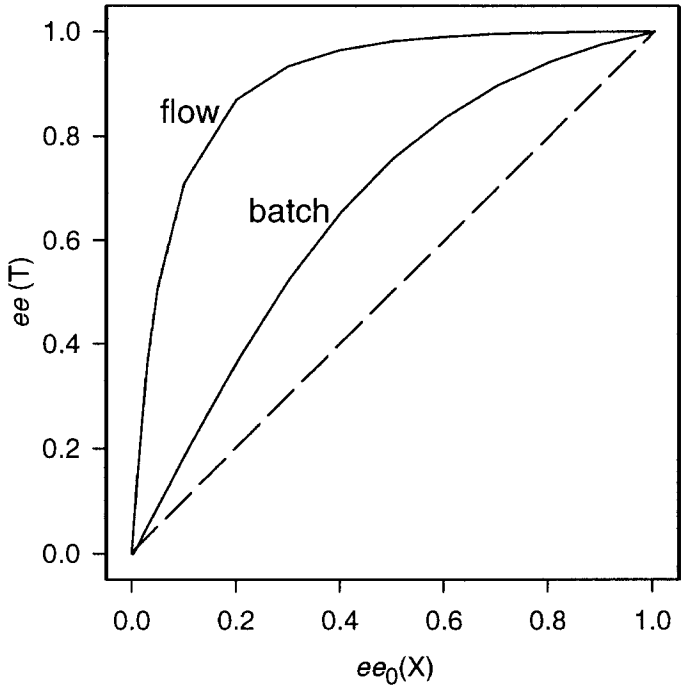


Figure 3. Simulation result demonstrating a positive nonlinear relationship between the product enantiomeric excess, *ee*(T), and the enantiomeric excess of the initially added or inflowing reactant mixture, ee_0(X). Upper curve: stationary values under flow conditions; lower curve: equilibrium values under batch conditions. $k_f = 1.8 \times 10^{-4}$ s^{-1}. The total concentration of reactants was always kept the same and is as given in Scheme 2. The broken line is a visual support for linearity.

amount of TLL that has been formed in the first reactor and that flows into the subsequent reactor does not react, i.e. cannot display any catalytic activity, because the substrate concentration of EL, NL, ED, and ND becomes too low. It is a typical situation of saturation kinetics in which – in this specific case – the increase of the catalyst concentration does not lead to any increase in further product formation. The TLL in excess simply dilutes the system, consequently leading to a decrease in ee_{ss}(T).

However, as shown in Figure 4, in the case in which the reactor coupling is assumed to occur in the presence of a kind of membrane filter that selectively permits the transfer of only the smallest molecules (EL, NL, ED, ND) into the subsequent reactor, the situation drastically changes.

In this case, stationary values in the product enantiomeric excess can climb to ee_{ss}(T) = 63.6% in the 8th reactor of the cascade when the scenario was started with an initial inflow into the first reactor of ee_0(X) = 0.9% of the reactant species. Here, the amplification effect is mainly a consequence of the chiroselective autocatalytic effect. This can be seen by the simultaneous variation of the reactant enantiomeric excess (see Figure 4). Both, the stationary enantiomeric excess in product, ee_{ss}(T),

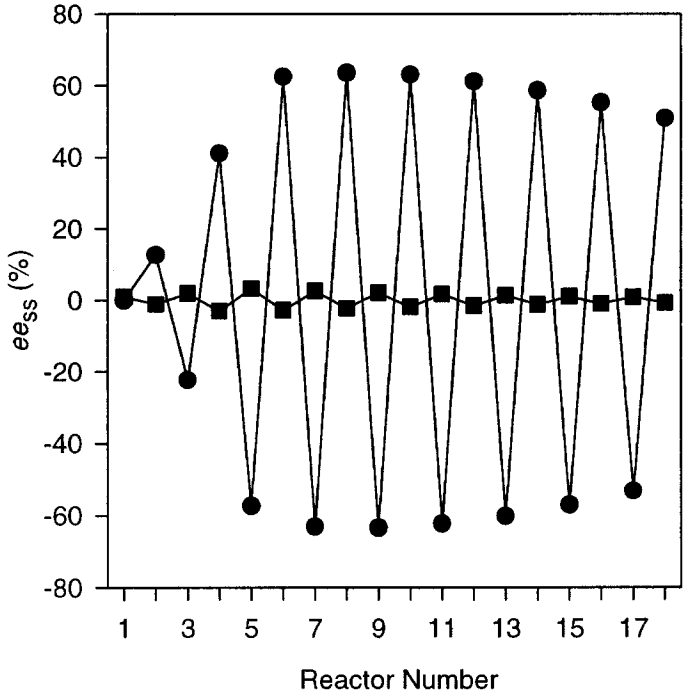


Figure 4. Prediction of the stationary enantiomeric excess (%) in the heterochiral Ghadiri-type system proceeding in a cascade of open flow reactors (see text). (●) Products, ee_{ss}(T) and (■) reactants, ee_{ss}(X). Inflow into reactor 1: [EL] = [NL] = 50.0 μM and [ED] = [ND] = 49.108 μM corresponding to ee_0(X) = 0.9%, $k_f = 1.8 \times 10^{-4}$ s^{-1}. Only transfer of EL, NL, ED, ND at their respective stationary concentrations from one reactor into the other (same k_f).

and in reactant, ee_{ss}(X), go through a maximum, however, at a different position in the cascade. The maximum in ee_{ss}(X) corresponds about to the position in the cascade in which an inflection point for the ee_{ss}(T) occurs. The decrease of the two can be explained by the repetitive consumption of the reactants from one reactor to the other since fresh solution enters exclusively into the first reactor of the cascade. Hence the concentrations of EL, NL, ED, and ND become increasingly smaller so that the feeding of the autocatalytic cycle (see Scheme 1) becomes more and more insufficient, which consequently weakens the chiroselective autocatalytic effect.

It is further interesting to note that in this cascade scenario a strictly alternate predominance of TLL and TDD product species can be observed ('flip-flop' effect), i.e. an oscillation in the sign of the optical rotation from one reactor the other. As, for instance, in the first reactor EL and NL are in excess over ED and ND and more TLL than TDD is produced, the consumption of the L-reactants occurs faster than that of the D-reactants, which in turn leads to an excess of ED and ND in the second reactor that form an excess of TDD over TLL, etc. Such a set-up can give rise to a spatial, i.e., chromatography-like, separation of enantiomers.

2.3. COMPUTATIONAL PROCEDURES

Model calculations, as described for the Chmielewski-type and the Ghadiri-type peptide self-replicators, were performed on a personal computer using Sa, 'Simulation & adjustment, version 2.0', which is a non-commercial program based on C^{++} and developed at the Laboratoire de IMRCP, Université Paul Sabatier, Toulouse, France. The general algorithm used for the numerical integration of the differential equations was based on a semi-implicit Runge-Kutta method and the basic optimization procedure using a nonlinear minimization algorithm for the adjustment of the rate parameters of the model to the experimental data have been already described elsewhere (Rivera Islas *et al.*, 2003a, b).

Open flow conditions have been simulated by adding flow terms (see Scheme 2) to the kinetic equations of the model considering each of the 34 involved species in the heterochiral reaction system. The reactor coupling has been considered to occur in each step of the cascade with the same flow rate parameter k_f and the transfer of species from one reactor into the subsequent one was thought to take place after stationary conditions have been already reached. For all cases, entirely homogeneous conditions (perfect mixing) were assumed.

3. Conclusion

In this article, we presented a further kinetic approach to describe artificial peptide self-replication based on a simplified but chemically realistic model scheme. The successful data fitting of the Chmielewski-type peptide self-replicator and, previously, of the Ghadiri-type system indicates that this model can serve as a basis for the kinetic analysis of peptide self-replicators in general.

It was shown that variations in the experimentally observed kinetics, caused by differences in the amino acid sequences of the catalytically active template products, are apparently neither due to changes in the underlying reaction network nor to changes in the stoichiometry of the association equilibria such as the formation of trimeric or tetrameric ligation complexes. The enhanced catalytic efficiency of the Chmielewski-type peptide self-replicator over the Ghadiri-type analogue can be traced back to major dissimilarities in the values of the involved rate constants, indicating a weaker product inhibition via the template dimerization and a stronger association of the single peptide fragments to the template product. These two effects are synergistically fueling the autocatalytic processing of the reaction system leading to the pronounced higher catalytic activity. The variations in the association equilibrium constants are certainly due to differences in the aggregative properties of the helical peptides, which would require a detailed structural analysis of the amino acid sequences that is out of the scope of the presented work.

It was further shown by the analysis of the heterochiral Ghadiri-type system that chiral implications in peptide self-replication can open a promising way to observe autocatalytically driven amplification of enantiomeric excess. In this respect

and depending on experimental verification, peptide self-replication could probably perform as the first model system in a laboratory experiment that combines the aspect of direct prebiotic relevance and the capacity for chiral amplification. Model predictions demonstrated that a small enantiomeric excess of the reactant species can lead to considerably higher enantiomeric excess of the product species. This enantiomeric amplification is due to a chiroselective autocatalytic effect and to a *meso*-type separation that both are intrinsic for the underlying dynamics of the reaction system. Amplification effects can probably be enlarged under open flow conditions, i.e., if the system is kept in a non-equilibrium state. Under these conditions, the flow rate parameter, regulating the net rate of flow of the involved species in and out of the reactor, has been shown to be sensitive for the obtainable stationary enantiomeric excess.

Finally, a cascade of open flow reactors equipped with a membrane filter at each of the outlets that exclusively allows the transfer of the small peptide fragments (EL, NL, ED, ND) from reactor to reactor results in the prediction of highly pronounced amplification effects. During the cascade scenario also the sign of the optical rotation alternates from one reactor to the other – an effect that should be probably additionally considered in general when discussing the origin of the sign of the *natural* optical rotation.

In resume, experimental proof is required if artificial peptide self-replication can indeed give rise to chiral amplification. It can be speculated that these effects – if confirmed – could be enhanced by especially designed peptides that, similar to the Chmielewski-type self-replicator, could display a stronger chiroselective effect by amplifying the autocatalytic efficiency of the underlying reaction network.

Acknowledgements

This work was supported by a bilateral Mexican-French cooperation project CONACyT-CNRS, grant E-130.1135/2001. T. B. and J. R. I. acknowledge additional financial support from the Mexican CONACyT research project program, Grant 34236-E.

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