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CHIRALITY

Spontaneous Mirror Symmetry Breaking in the Aldol Reaction and its Potential Relevance in Prebiotic Chemistry

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Abstract The origin of the single chirality of most biomolecules is still a great puzzle. Carbohydrates could form in the formose reaction, which is proposed to be autocatalytic and contains aldol reaction steps. Based on our earlier observation of organoautocatalysis and spontaneous enantioenrichment in absence of deliberate chiral influences in the aldol reaction of acetone and p-nitrobenzaldehyde we suggest that a similar effect might be present also in the aldol reactions involved in gluconeogenesis. Herein we show that reactant precipitation observed in our earlier reported experiments does not affect the asymmetric autocatalysis in the aldol reaction we studied. We explain the phenomenon of spontaneous mirror symmetry breaking in such organocatalytic homogenous systems qualitatively by non-linear reaction network kinetics and classical transition state theory.

Keywords Aldol reaction · Formose reaction · Absolute asymmetric synthesis · Spontaneous mirror symmetry breaking · Homochirality

Introduction

The origin of life is closely related to the problem of the origin of biological homochirality (Calvin 1969; Bonner 1994; Cintas 2002). Only one enantiomeric form of amino acids or sugars dominates in the polymeric biomolecules. Did life start with both forms of chiral handedness—with one form becoming extinct ? Or did the choice of a single handedness predate the advent of replicating and information-carrying biomolecules ? Is nature's prevailing choice of L-amino acids and D-sugars deterministic or accidental ? Ever since the groundbreaking work of Pasteur (1848), this conundrum posed tantalizing questions for generations of chemists. Polymerisation of amino acids in β-sheets (rather than α-helices) could conceivably have generated enantioenriched ensembles of chiral polymers (Rubinstein

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et al. 2008). When, alternatively, the single chirality of RNA or DNA (with sugar backbones) on one hand, and of proteins (comprising in the most general form of both α-helix and β-sheet domains) on the other hand, stemmed from a chiral pool of already enantioenriched monomeric amino acids and sugars, we are faced with the question which came first, i.e. whether the single chirality of amino acids might have caused the single chirality of sugars or vice versa.

Pizzarello and Weber first suggested that the gluconeogenesis might have proceeded stereoselectively in presence of catalytically active amino acids (Pizzarello and Weber 2004; Weber and Pizzarello 2006). The connection between the naturally mostly occurring L-amino acids and of the predominantly naturally occurring D-sugars has been established for the case of serine octamers (Nanita and Cooks 2006) and it was shown that racemic amino acid solutions can be kinetically resolved by a small enantiomeric excess in the carbohydrate (Cordova et al. 2006). It might be rewarding to consider a further possibility: the homochirality of biomolecules could have originated endogenously from the carbohydrates, rather than from their interaction with amino acids (Toxvaerd 2005).

It appears natural to assume that the single chirality of monomeric sugars and amino acids did not evolve concurrently or even in competition. It is commonly assumed that carbohydrates originated from the condensation of formaldehyde in the formose reaction (Butlerov 1861; Breslow 1959; Morozov 1991; Orgel 2000). This reaction is by no means clean and gives a variety of sugars with a maximum for the trioses, tetroses or pentoses, depending on the reaction conditions (Morozov 1991). The reaction, assumed to be a sequence of aldol reaction (Breslow 1959), aldose-ketose isomerizations and (possibly) aldol condensation steps, is known to be autocatalytic (Socha et al. 1980). Non-linear autocatalytic reaction networks, like the well-known Frank mutual inhibition mechanism (Frank 1953) or hypercompetitive mechanisms (Decker 1975), are known to be able to reproduce qualitatively the asymmetric amplification and spontaneous mirror symmetry breaking that could have resulted in a homochiral pool of precursors for biomolecules.

It has been shown theoretical by molecular dynamics simulations (Toxvaerd 2005), employing collision kinetics in keto-enol isomerizations in combination with the thermodynamic scheme of chiral discrimination, that racemic mixtures of glyceraldehydes may deracemize at high temperatures and particle densities via reaction step direction dependent activation barriers. In contrast, non-linear enantioselective autocatalytic reaction schemes might principally explain symmetry breaking and asymmetric amplification also in diluted solutions which could have existed in the primordial oceans.

"Asymmetric autocatalysis", in which the chiral product automultiplies (Alberts and Wynberg 1989), may be key to these symmetry-breaking processes. It was first proposed by us as a possible mechanism of chirality induction in organocatalytic reactions (Mauksch et al. 2007a, b), but has not yet been proposed for the formose reaction. Asymmetric autocatalytic reactions might even be capable to exhibit spontaneous mirror symmetry breaking when combined with a non-linear underlying reaction network mechanism (Frank 1953). Soai's et al. 1995' discovery of an irreversible asymmetric autocatalytic reaction of a pyrimidyl-5-carbaldehyde with a Zn organyl, appears to be a first example (Soai et al. 1995). In the strict sense, "asymmetric autocatalysis" implies that catalyst and product are identical. Later studies revealed that the Soai reaction is second—not first—order in the aldehyde concentration (Buono and Blackmond 2003), though. Blackmond and Brown further observed in heat flow measurements, that the racemic catalyst has only half the activity compared to the enantiopure catalyst and explained this by the proposal that the actually active catalytic species in the Soai reaction should be a homodimeric chelate, rather than the monomeric alkoxide product or the heterodimer and based on the concept of

unspecific mutual antagonism (Blackmond et al. 2001). This interpretation was further corroborated by a recent computational investigation of the mechanism (Schiaffino and Ercolani 2008).

While Zn ions are rather common in the active centers of enzymes, organometallic reactions which require organic solvents are unlikely to have played an important role on prebiotic earth where water was probably ubiquitous. We wondered therefore whether also purely organic and reversible asymmetric reactions could exhibit product catalysis and spontaneous mirror symmetry breaking and we were seeking for an experimental demonstration in the laboratory. The first examples for such organoautocatalysis, asymmetric Mannich and aldol reactions, i.e. carbon–carbon bond forming transformations and which could be conceivably involved in biosynthetic pathways of sugars or amino acids, have been reported by us in 2007 (Mauksch et al. 2007a), shortly followed by our finding that the same reactions are even able to exhibit "spontaneous mirror symmetry breaking" (Mauksch et al. 2007b). The underlying mechanism for this effect remains still unclear, though (Fig. 1).

That we were able to demonstrate "spontaneous mirror symmetry breaking" in a closed *reversible* and presumably already well-understood reactive system, was met with some doubt (Blackmond 2009), despite the fact that we explained the phenomenon as occurring under kinetic control and stated that the final outcome must necessarily be racemic because of the second law of thermodynamics and the principle of microscopic reversibility, which both in effect demand that enantioimbalances must be reduced when the thermodynamic equilibrium is approached—in addition to the static preference of the racemic state because of the unavoidable mixing entropy. Herein we present results of new experiments on the aldol reaction of acetone and p-nitrobenzaldehyde **2** that confirm our earlier findings, but are also aimed at elucidating the role of the sometimes observed reactant precipitation in the aldol reaction (Mauksch et al. 2007b). It is also explained that the combination of classical transition state theory in conjunction with non-linear feedback reaction network mechanisms on the basis of classical rate equation theory is necessary but also sufficient to understand qualitatively the symmetry breaking and asymmetric amplification observed in homogenous systems.

Results and Discussion

Experimental Results

We set out from repeating our earlier experiments in which we observed spontaneous product enantioenrichment up to about 50% in an aldol reaction in acetone/DMSO, starting from achiral reactants and without chiral auxiliaries—i.e. constituting a rare example of a true "absolute asymmetric synthesis". We varied the initial concentration of p-nitrobenzaldehyde, the reaction volume and the corresponding amount of DMSO, added

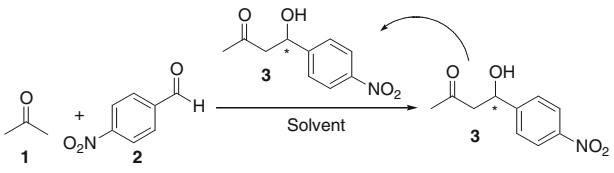

Fig. 1 Autocatalytic aldol reaction of p-nitrobenzaldehyde and acetone

to increase reactant solubility (see Table 1). To prevent acetone from evaporating, we sealed the reaction tubes with paraffin. As a result, we found that the final ee value of **3** (after work-up) ranges from 0.29 (*R*) to 14.33 (*S*). Because our work-up procedure does involve the purification of the product mixture (after removing the excess reactant) on DC plates (see Experimental section) some caution is justified in interpretation of small ee values. Earlier, we found for the Mannich reaction, that self-disproportionation of enantiomers (Soloshonok 2006) might occur in this purification procedure leading to the unreliability of ee values below about 2% ee. But even if these small values are discarded, eight values in Table 1 (entries 1,4–6 and 11–14) lie significantly above this threshold.

All reaction mixtures had been fully homogenous when the reaction was started, but in some runs (1–4, 11–16) precipitation (identified as reactant **2**) has been observed and sometimes the final reaction mixtures before the reaction was stopped more resembled a suspension rather than a homogenous solution. Because p-nitrobenzaldehyde crystallizes in a monoclinic chiral space group $P2_1$ (Jackisch et al. 1989), we wondered whether the observed symmetry breaking could have been influenced by the precipitation, e.g. by stereospecific attachment of enantiomers to the surface of the reactant crystals. Hence, in a separate series of experiments, we determined the solubility of the reactant in the solvent mixture (0.175 g ml^{-1}) and prepared saturated solutions of p-nitrobenzaldehyde to which we added different amounts of excess aldehyde (Table 2). The product enantiomeric excess obtained, ranges here from 0.56% (*S*) to 17.2% (*R*). Again discarding the smallest values (below 2% ee), we achieved significant spontaneously generated enantiomeric excess in three experiments (entries 3, 5 and 7). The ee values are obviously not correlated to the amounts of reactant added. To see the effect of increased available crystal surface area through crystal crushing, we added glass beads to the vigorously stirred (at 300 rpm)

Table 1 Enantiomeric excess in different runs of the aldol reaction of p-nitrobenzaldehyde (**2**) and acetone (**1**) without chiral auxiliaries or reactants

Reaction no.	Reaction volume (ml)	Added DMSO (volume %)	Nitrobenzaldehyde amount (mg)	ee% (configuration)[a]
1	0.3	3.3	60.5	14.3 (S)
2	0.3	3.3	60.5	2.7 (S)
3	0.3	3.3	60.5	2.3 (S)
4	0.3	3.3	60.5	3.2 (S)
5	2.5	3.3	500	3.2 (R)
6	2.5	3.3	500	4.1 (R)
7	2.5	3.3	500	0.9 (R)
8	2.5	3.3	500	1.7 (R)
9	2.5	3.3	500	0.3 (R)
10	2.5	3.3	500	1.2 (R)
11	0.15	2.6	30.2	3.6 (S)
12	0.15	2.6	30.2	7.3 (S)
13	0.15	2.6	30.2	3.6 (S)
14	0.15	2.6	30.2	7.4 (S)
15	0.15	2.6	30.2	1.2 (S)
16	0.15	2.6	30.2	1.7 (R)

[a] Enantioselectivities were determined by chiral-phase HPLC analysis (Daicel Chiralpak AS) in comparison with authentic racemic material

Table 2 Enantiomeric excess in different runs of the aldol reaction of p-nitrobenzaldehyde (**2**) and acetone (**1**) in saturated solutions (1 ml volume with 3.3 vol% DMSO) with extra added crystalline aldehyde

Reaction no.	Nitrobenzaldehyde additional amount (mg)	Glass beads[a]	ee % (configuration)
1	200	–	0.6 (S)
2	200	2.4 g	1.3 (S)
3	400	–	17.2 (R)
4	400	2.4 g	1.7 (R)
5	600	–	2.5 (R)
6	600	2.4 g	1.0 (R)
7[b]	0	–	7.9 (R)

[a] Glass beads (200×300 μm) were added in some experiments to the stirred solutions in order to increase the available crystal surface area

[b] Control experiment without extra reactant

suspensions (entries 2, 4 and 6). The results showed, however, evidence for an apparently negative correlation between final ee value and presumed crystal surface area for a given amount of excess reactant aldehyde.

We concluded therefore that the spontaneous mirror symmetry breaking occurs in the homogenous solution phase and is not caused or assisted by the presence of excess crystalline aldehyde, e.g. through adsorption phenomena. It should be noted though that all experiments were stopped after 10 days and that a continuous monitoring of enantiomeric excess in solution would be necessary to unveil actual correlations. This is because the asymmetric amplification is explained as a temporary phenomenon under kinetic control (Mauksch et al. 2007b), and the enantiomeric excess could pass through its maximum value before the reaction is stopped.

The enantioenriched aldol product itself (with 76% ee), when employed as a product catalyst in the aldol reaction, is able to give highly enantioenriched newly formed product of the same absolute configuration and with up to 75% ee (Mauksch et al. 2007a). This shows that both the enantioselectivity and the chirality induction (i.e. stereospecifity) are remarkably high. When we instead used as "catalyst" a compound structurally similar to the aldol product with acetone (namely the product, **4**, of the reaction with cyclohexanone, **5**), the achievable enantiomeric excess was much lower, 9.0% ee, and only traces of product were detected (Fig. 2)! With cyclohexanone as ketone instead of acetone and in

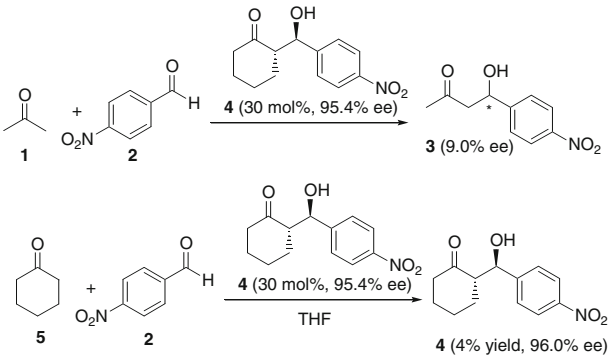

Fig. 2 Aldol reaction of acetone with p-nitrobenzaldehyde, catalyzed by the aldol product **4** of the aldehyde **2** with cyclohexanone **5**

tetrahydrofuran as solvent we obtained an enantiomeric product excess of 96.0% and 4% yield of newly formed product **4** with 30 mol% product catalyst loading at 95.4% ee. These results underline the importance of structural resemblance of product and catalyst, indicative of a template directed (self-replicating) mechanism. Because all reaction steps in our mechanism are reversible, reactant should also form from pure product solutions in retro-aldol reactions (Mauksch et al. 2007b). This has been investigated e.g. for the aldol reaction shown in Fig. 1, where we have even found some evidence that a racemic product solution reverts faster to the reactant than an enantiopure product solution. This made us wonder whether a "recycling" reaction pathway different from either the mere reversal of the autocatalytic step or the reversal of the slow uncatalyzed product formation could exist (i.e. by some sort of "synergistic" interaction of the enantiomers).

Intriguingly we observed for the Mannich reaction (Mauksch et al. 2007a, b) that the achievable yields with initially added enantiopure product catalysts were about three times the yields obtained with the same amount of racemic catalyst initially added (with 15 mol% loading). It appears that the rate of product formation is affected by the enantiomeric purity of the added product catalyst, providing important information on the mechanism of the asymmetric transformation. We reasoned therefore that dimer equilibria involving the product catalysts (e.g. R + S ↔ RS) could be involved in the enantioselective transformation.

At first glance, the synthetic value of such findings appears to be low, also because the yields achieved (and, hence, the chiral productivity) were very small (usually 1–10% when no catalyst was used and 3-27% when product catalyst was employed). However, the "spontaneous random generation of enantiomeric excess" could be a rather widespread phenomenon in reactions which allow for specific attractive product–substrate interactions, e.g. by hydrogen-bonding or similar secondary interactions or even by covalent bonding. This could further our understanding of processes in asymmetric transformations and might also be of future synthetic value, once the asymmetric amplification effect can be sufficiently controlled and its anticipated generality proven. Moreover, Mannich and aldol reactions could, in principle, both proceed also in water (Dong et al. 2007; Hayashi et al. 2005), which might appear important in the "origin of life" context, in the light of the probably aqueous prebiotic world.

Non-linear autocatalytic Reaction Networks

Whether matter exchange with the environment is allowed or not should be distinguished clearly from the nature of the reaction network that operates inside the system. The Frank network mechanism allows the spontaneous generation of chirality in homogenous closed *or* open systems through the combination of asymmetric autocatalysis (automultiplication of a chiral product) and mutual inhibition (by the recombining interaction of the product enantiomers), implying the (not necessarily irreversible) formation of achiral heterodimers as an energetic sink in the mechanism (see Fig. 3a). Notably, the heterodimers might not even necessarily be achiral (Schiaffino and Ercolani 2008). It should also be noted that the distinction of systems as "open" or "closed" to matter exchange with the environment is somewhat artificial, because any small enough subsystem of a closed system could also appear as an open system for all practical purposes. However, while an open flow system can be kept in a permanent non-equilibrium (i.e. steady) state by imposing constant thermodynamic restrictions, a closed system can only be *temporarily* in such a slowly changing (quasi-steady) state and more or less slowly reverts back to thermodynamic equilibrium. Naturally, Charles Frank anticipated in 1953 that a demonstration of mirror

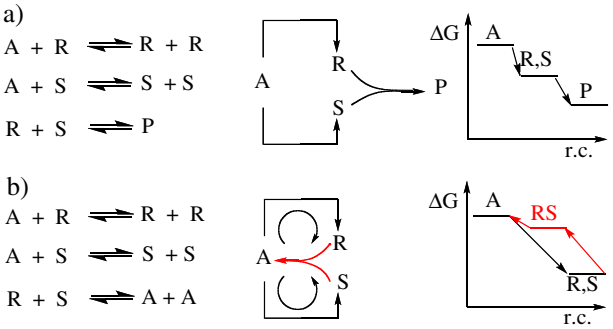

Fig. 3 Comparison of two Frank-type mathematical model mechanisms for spontaneous enantioenrichment. Open-network (original) Frank mechanism (**a**) and closed-network Frank mechanism (**b**) with their schematic reaction energy profiles (A = prochiral reactant). Mechanism (**b**) contains cyclic fluxes through coupling of the *non-spontaneous* reactant recycling step (*marked in red*) to external exergonic reactions and a RS-heterodimer *higher* in energy than the monomer, while mechanism (**a**) implies an "energy cascade" with heterodimer P *lower* in energy than the monomer. In both mechanisms (**a**) and (**b**), *monomeric* product catalysts result from the autocatalytic steps

symmetry breaking by his non-linear network mechanism in a "laboratory flask" might not be impossible!

A distinction of open vs. closed *reaction networks* has also to be made. Both open or closed networks might operate conceivably under either closed or open system conditions. We recently compared a proposal for a closed-network version of Frank's mechanism (Mauksch et al. 2007a, b) with the original open-network Frank mechanism (see Fig. 3). In contrast to an open network, a closed-network mechanism implies cyclic matter fluxes in the network by the overlay of two different reaction pathways that connect reactants and products and which could principally result in near complete homochirality through recycling of the less abundant enantiomer product—provided the thermodynamic restrictions permit this. This would conceivably allow for *complete* symmetry breaking and asymmetric amplification in a one-pot reaction without a sequential batch reaction protocol as in the Soai reaction (Saito and Hyuga 2004). In contrast, a non-cyclic mechanism is unlikely to give 100% enantiomeric excess without such sequential work-up procedures, because a recycling path for the minor enantiomer is absent.

As we explained in our earlier work (Mauksch and Tsogoeva 2008), symmetry breaking in non-linear Frank-type reaction networks in which mutual inhibition and only linear autocatalysis are combined, implies a bistability of the system: at the beginning of the reaction, the reaction mixture evolves as a *racemic* system when the initial conditions had been nominally racemic, until a bifurcation point (B.P.) is reached from which the system rapidly evolves from a racemic into a symmetry broken state (Fig. 4). This asymmetric amplification can of course only be of temporary nature, though, in a reversible system that is *closed* to matter flow and in absence of a permanent irreversible flow of energy through it: once the ee maximum is reached, the reversibility and the symmetry of the reaction network demands, that the system, which is so displaced from its racemic equilibrium state, will exhibit now a gradual monotonous (exponential) erosion of enantiomeric excess. In contrast, *nonsymmetric* reaction networks (with a dissymmetric matrix of stoichiometric coefficients) might even support chemical oscillations in closed reversible systems (Gray et al. 1988). We found that the total amount of product catalyst (i.e. the concentration [Q]=[R] + [S]) does not change when passing from the racemic into the symmetry broken state if the accumulation of the more abundant autocatalyst is limited through a constant outflow

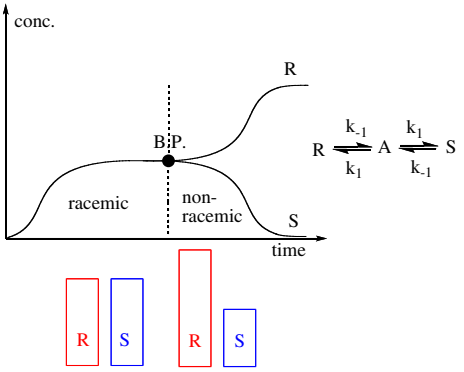

Fig. 4 Schematic depiction of bifurcation, e.g. in open or closed mutual inhibition (Frank-type) networks. The enantiomeric excess is a function of time and can be factored into a slower racemizing part ee_{rac} (decreasing with time) and a faster deracemizing part ee_{derac} (increasing with time). Racemization might occur via the reactant in reversible processes

keeping the total amount of autocatalyst constant (Mauksch and Tsogoeva 2008). In this case, the symmetry breaking involves a symmetric pitchfork bifurcation without continued product catalyst autoamplification and is entropic, instead of an enthalpy driven process (Plasson 2009).

The time dependent function ee(t) for both Frank-type models (Fig. 3a and b) in the region after the bifurcation point can obviously be factorized phenomenologically into a racemizing part (ee_{rac}) and a deracemizing part (ee_{derac}): $ee(t) = ee_{rac} * ee_{derac}$ ("*" denoting the convolution of the two functions). Both racemization (either via the reactant: R ↔ A ↔ S, e.g. when the "autocatalytic" steps are reversible, or directly: R ↔ S) and deracemization begin concurrently and immediately once the bifurcation point is reached. As the racemization depends e.g. on the retro-aldol step (when direct enantiomerization and background reaction can be neglected) with rate constant k_{-1} and the deracemization depends on k'_1 (the rate constant of the enantioselective autocatalytic step, k_1, with the reactant concentration absorbed) and with $k_1 \gg k_{-1}$ (Fig. 4), racemization is much slower than deracemization (Crusats et al. 2009), resulting in a local maximum in ee(t), which may be sizable for appropriate rate constants. The influence of the time dependent order parameter ee(t) on the rates of both racemization and deracemization is neglected here for simplicity: the deracemization, for example, is fastest, when (for a given total concentration [Q]), the autocatalyst is enantiopure (ee=100%), because the difference in concentration of the more abundant enantiomer to the less abundant form grows most rapidly then. However, racemization is fastest, too, when the displacement of the reaction mixture from the racemic (kinetic and thermodynamic) equilibrium state (Ribo and Hochberg 2008) is maximal (i.e. again for ee = 100%), because the enantiomerization of the more abundant into the less abundant enantiomer is then not counterbalanced by an enantiomerization in the reverse direction. Hence, when the product forming reaction steps are irreversible and the product is optically stable (as e.g. in the Soai reaction), racemization cannot occur. The kinetic instability of the homochiral state may also be deduced from the symmetry of the reaction network.

The equilibrium constants of conceivable (even irreversible) reactions (1) A + R → 2 R (linear autocatalysis) and e.g. (2) 2 A → R + S (non-catalytic disproportionation) in a cyclic mutually inhibitive closed-network mechanism are trivially not independent of each other:

for the reaction free energy holds $\Delta G_2 = 2\ \Delta G_1$. As a consequence, a rate constant dependency, taking into account the reverse steps, follows (Blackmond 2009). Blackmond, however, also assumed that the "closed-network Frank mechanism" proposed by us would be equivalent to a "closed matter system" (i.e. in which conservation of mass, e.g. [A]–[R]–[S]–½ [RS]=0, is observed, and no other reactions than those present in the Frank-type scheme are present). Under these implicit assumptions (and when the formation of the autocatalyst is exergonic), the "closed-network mechanism" was shown to give only asymmetric depletion, in accord with the law of energy conservation. "Microscopic reversibility", an addendum to the first two laws of thermodynamics requiring detailed balance as a necessary condition and which is not assumed to hold in open non-equilibrium systems (Qian and Wang 2006) was invoked by Blackmond to explain this (Blackmond 2009). However, Blackmond's treatment may appear restrictive and does not take into account the likely presence of side reactions or follow-up reactions (e.g. the quasi-irreversible aldol condensation step) inside the *experimental* closed matter system and which can conceivably couple to individual reaction steps in a recycle mechanism (Fig. 3b). It was already shown that such coupling to exergonic reactions *external* to the cyclic network but *internal* to the closed matter system may formally allow the spontaneous ee generation and amplification (Plasson 2009). Indeed, we have found several of these side reaction products in the aldol product mixture before purification, indicating that the system must be much more involved then had been assumed in a simplified model system treatment (Blackmond 2008; Blackmond 2009).

Hence, the Frank model(s) seem to qualitatively reproduce the observed symmetry breaking and asymmetric amplification in our experiments, e.g. for the aldol reaction. However, it should be noted that this mechanism hinges on several implicit assumptions which may collide with mechanistic considerations. First, the enantioselective autocatalysis is assumed as stoichiometric as in a one-step reaction where the rate determining step necessarily coincides with the configuration determining step. Actual organic reactions are mostly multi-step, though. Moreover, the Frank mechanism doesn't account for the specific nature of the chiral induction and 100% stereospecifity is implied in the enantioselective reaction.

Mechanistic Considerations

The Soai reaction is much better investigated than our organocatalytic examples for asymmetric autocatalysis. A comparison might therefore yield valuable insights. That for Li or Mg alkoxides a similar behaviour, as compared to that of certain Zn alkoxides formed from appropriately substituted aromatic aldehydes, is unknown, could be likely due to the different coordination chemistry of Zn^{2+} as compared to that of the "harder" Mg^{2+} or Li^{+} ions. Indeed, while enzymes containing Zn ions in the active center are ubiquitous, enzymes containing Li or Mg in the active center are not known. This points—for chemical reasons—to an explanation on the basis of dimeric or higher Zn alkoxide aggregates where the free coordination sites at the metal center are occupied by alkoxy or other O-ligands. However, it appears that the "specific mutual inhibition"—involving monomeric catalytic species and reversibly or irreversibly formed heterodimers of lower energy than homodimers, to siphon off a more than proportional part of the minor enantiomer, and as invented by Frank, opposed to "unspecific mutual inhibition" with homodimers in statistical equilibrium with heterodimers of similar energy (Kitamura et al. 1995), cannot be discarded off-hand, because homodimers could of course be considerably less stable than heterodimers, which could conceivably also form faster than the homodimers (Rivera Islas et al. 2005).

In contrast, we have no such coordination "sites" available in our organocatalytic version of "absolute asymmetric synthesis". Dimer formation can therefore only be the result of secondary interactions, or, alternatively, covalent binding. The great success of organocatalysis in homogenous catalysis clearly demoted earlier anticipations that secondary interactions are too "weak" to bring about a significant catalytic effect or chirality induction in the absence of ligand sites at a metal center. The product catalyst may act instead as a template for the formation of more product in hydrogen-bonded transition states. This has important consequences that should be taken into account when mechanistic proposals are made. The autocatalysis can hardly expected to be more than linear and the catalytic species must therefore be the monomeric product itself. Because such a purely linear autocatalysis (in contrast to the quadratic or higher-order autocatalysis suggested for the Soai reaction) cannot yield symmetry breaking or asymmetric amplification (Hochstim 1975), we came up with the proposal (Mauksch et al. 2007b) of a mutual inhibition mechanism involving heterodimer formation (see Fig. 3).

It should be noted that—in this particular type of mechanism—product dimers would not form only *after* the autocatalytic step in a separate reaction, their formation in our organocatalytic examples is rather a consequence of the template directed product formation in the autocatalytic step itself. Heterodimers (RS) would form e.g. as a result of passing through anti-selective transition state structures, while homodimers (SS and RR) form from selective transition structures. These dimers are in equilibrium with the monomers which could act as catalysts in further transformations.

Is there experimental or computational evidence for such dimer formation in the actual organoautocatalytic transformations ? While a mass spectrum taken from the aldol reaction mixture after 10 days did not show a peak indicative of dimer formation, at least our earlier quantum chemical (DFT) calculations for the Mannich reaction suggested that both homo- and heterodimers could exist in the gas phase, albeit unexpectedly both about 3 kcal mol^{-1} *higher* in energy compared to the monomers at the computational level employed, with the heterodimers slightly preferred energetically (by 0.6 kcal mol^{-1}) and with respect to the homodimers. When the computational level employed (B3LYP/6-31G) is applicable here, these results suggest that a "classical" Frank mechanism (Fig. 3a) might not apply in these organoautocatalytic reactions.

The distinction, whether a thermodynamic or a kinetic—e.g. a closed or open network mutual inhibition—mechanism applies in the description of these organocatalytic examples for absolute asymmetric synthesis, can only be made on the basis of a still lacking elucidation of the reaction mechanism. The validation of the energetic aspect—i.e. the reaction profile—is therefore very important. In the case of an open-network (original) Frank mechanism (Fig. 3a), the heterodimer (e.g. being strictly optically and catalytically inactive) has to be *more* "stable" than the catalytic chiral monomer products to achieve a reduction in concentration of the competing antipode autocatalyst and accumulates in the system as an "end product" (resulting of course in a lowered final ee after work-up, in case the heterodimer would not resist the work-up conditions - and when it is not removed as a side product from the reaction zone, because some of the minor enantiomer is of course "conserved" in form of the RS-dimer during the reaction).

In contrast, the linear asymmetric autocatalytic mechanism proposed by us naturally implies that not only minima on the potential energy surface, but also transition structures with the homo- or heterodimer geometry both exist. It should be noted, that the products of the autocatalytic step necessarily should have a geometry similar to that of their respective transition structures. When the enantioselective transition structures are energetically preferred, more homodimers than heterodimers will form under kinetic control, i.e. faster.

Hetero- and homodimers are diastereomeric species that might show different chemical reactivity or kinetic stability. Hypothetically, homodimers could e.g. more facially decay into monomeric catalysts than heterodimers. The (accidentally) more abundant of the two possible homodimers would consequently release *more* monomeric catalysts in the same time interval, assisting in the formation of still more homodimers of the same absolute configuration and so forth, while the more inert (and/or thermodynamically preferred) RS-dimers could have a much slower lifecycle. A thorough quantum-mechanical investigation of the possible reaction mechanism and the transition structures involved is therefore called for to answer these questions.

Conclusions

Our new experiments on the aldol reaction of acetone or cyclohexanone and p-nitrobenzaldehyde confirm our earlier conclusions, that spontaneous mirror symmetry breaking and asymmetric amplification are possible in closed reversible reactive systems and that an organocatalytic version of an absolute asymmetric synthesis can be demonstrated. We proposed the first example of an asymmetric autocatalytic aldol reaction and presented here for the first time evidence that a precipitation of reactant observed in course of these reactions is not correlated with the achievable enantiomeric excess when the experiments are stopped after ten days. In the discussion we explained herein that classical transition state theory in combination with a non-linear reaction network mechanism is necessary, but also sufficient, to qualitatively understand symmetry breaking and asymmetric amplification in such organoautocatalytic reactions like the asymmetric aldol reaction and in the absence of chiral reactants or auxiliaries—even though the details of the mechanism are still unclear. For a quantitative understanding of the experimental results reported herein, the mathematical Frank model does not seem to be fine-grained enough to account for the relevant aspect of chemistry.

Different types of aldol reactions are known to be an integral part of the formose reaction which yields a variety of carbohydrates from “condensation” of formaldehyde. We cannot preclude therefore that aldol reactions as those occurring in the formose reaction could also be subject to asymmetric autocatalysis and could conceivably have been involved in the processes that engendered the homochirality of biomolecules, if originated from a pool of precursors of a single chirality.

Experimental

General

Solvents were purified by standard procedures and distilled before use. Reagents obtained from commercial sources were used without further purification. TLC chromatography was performed on precoated aluminium silica gel SIL G/UV254 plates (Marcherey, Nagel & Co.). Flash chromatography was performed using silica gel Merck 60 (particle size 0.040–0.063 mm). ^{1}H NMR spectra were recorded in CDCl3 with Varian Unity 300. HPLC measurements were performed using Agilent 1200 Series enginery: Vacuum Degasser G1322-90010, Quaternary Pump G1311-90010, Thermostated Column Compartment G1316-90010, Diode Array and Multiple Wavelength Detector SL G1315-90012, Standard and Preparative Autosampler G1329-90020 and Agilent Chemstation for LC software.

General and representative procedure for the reaction between *p*-nitrobenzaldehyde (2) and acetone (1) (e.g. entries 1–4, Table 1; concentration of p-nitrobenzaldehyde: 1.32 mol/l):

To a stirred solution of *p*-nitrobenzaldehyde (60.48 mg, 0.39997 mmol) in acetone (294 μL), DMSO (10 μL, 3.3 vol%) was added and the mixture was stirred with 300 rpm at ambient temperature for 10 days. The reaction mixture was diluted with CH_2Cl_2, washed with water to remove the DMSO and organic layer was dried over $MgSO_4$. The organic phase was subjected directly to column chromatography over silica gel (petrol ether/ ethyl acetate 3:1) to remove the aldehyde. The product and impurities were eluted with ethyl acetate, evaporated and purified further by thin layer chromatography (petrol ether/ ethyl acetate 3:1).

The enantiomeric excess of the product was determined by chiral HPLC analysis (Daicel Chiralpak AS, 2-propanol/hexane 20:80, flow rate 1.0 ml/min, λ = 254 nm, 10°C): t_R (R configuration) = 22.1 min; t_R (S configuration) = 35.7 min. ^{1}H NMR (300 MHz, CDCl3): δ= 8.2 (d, 2H), 7.53 (d, 2H), 5.24 (m, 1H), 3.58 (br s, 1H), 2.84 (m, 2H), 2.20 (s, 3H).

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