Ab initio study of chiral discrimination in alanine

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Chiral discrimination of intermolecular interaction between a pair of alanine is studied using *ab initio* theory (HF/6-311++G) with rigid geometry of molecules and also with relaxed geometry of the same using semi-empirical (PM3) level of theory. The energy optimizations of the homochiral pair (L-L) and heterochiral pair (D-L) of alanine molecules are carried out with variation in distance and orientation between the molecules. The study reveals interesting discrimination as a function of distance and orientation without any use of parameters for both rigid and relaxed optimization. The implication of the observed orientation dependence of the preferred homochirality in the process of peptide biosynthesis is discussed.**

Keywords: Alanine, chiral discrimination, intermolecular interaction, peptide biosynthesis.

CHIRAL discrimination continues to be one of the active areas of research for several years due to the importance of chirality at all levels of biological architecture and its functionality. While nature preferred homochirality through evolution, biomimetic molecules showed both homochiral preference (interaction of the same type of enantiomers are preferred over those between mirror-image isomers) and heterochiral preference (interaction of mirror-image isomers are preferred over those between same type of enantiomers)[1]. Effective pair potential studies indicated that the mutual distance and orientation of the chiral molecules could play a significant role in determining the degree of discrimination as well as homo- or heterochiral preference[1].

Peptide biosynthesis is an important biochemical reaction where chiral discrimination is important. The mechanism of peptide biosynthesis is a fundamental and still debated topic in molecular biology[2–12]. However, it is now accepted that the general mechanism of the reaction has a major step as the peptidyl transferase reaction, which takes place in the large subunit of the ribosome[2–6]. The ribosome possesses three tRNA-binding sites denoted as A (amino acyl), P (peptidyl) and E (exiting). In this reaction, the α-amino group of the amino acyl (aa)-tRNA at A-site makes a nucleophilic attack at the carbonyl carbon of the ester bond of the peptidyl (pept)-tRNA at P-site[12], and forms an intermediate, which subsequently forms the peptide bond (see Figure 1 *a*) and the polypeptide chain length grows.

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Ribosomes exclusively accept the L-isomer and accurately reject the D-isomer, which is a surprising choice considering the subtle difference between the two isomers (they only differ in the spatial arrangement of the groups attached to the chiral centre and the interaction energies of D–D and L–L pair differ only by the order of the thermal energy, k_BT). Considering the small enantiodifference, it seems probable that the D-amino acid can be incorporated as well into the growing polypeptide chain during peptide biosynthesis (to form a D–L pair) without any significant energetic disadvantage over naturally exclusive L–L synthesis. D-amino acid is isolated from amphibians and other invertebrate species like snails, cray fish and lobster. However, the enzymatic actions of such peptides containing D-amino acids are different from the usual peptides with L-amino acids or possibly have no activity[13]. Summarily, incorporation of D-amino acid is not identically accepted into the natural peptides, unlike the incorporation of L-amino acids. It is also important to note that despite the fact that tRNA normally carries L-amino acids, minute presence of D-amino acid can lead to mistakes[9], which will result in diminished or changed biological activity. The question of how the L-amino acid is exclusively preferred over D-amino acid is yet to be answered[8,14].

The orientation dependence of the intermolecular interaction profile of the amine group at the A site and the carbonyl carbon at the P site is extremely important. The A to P motion is rotatory and is highly stereo-specific in order to successfully form a peptide bond. The reaction is extremely fast and highly accurate[10,15]. The peptidyl transferase centre is an arched region, which is suitably designed for A–P rotatory motion. It is indicated that guided by RNA scaffold along an exact pattern, the rotatory motion leads to stereochemical arrangement optimum for peptide-bond formation[9]. Consequently, it is an important question whether chiral discrimination exists in the process of intermolecular interaction between L–L and D–L as function of mutual rotation. It may be noted that detailed and accurate information of the orientation dependence of interaction energy for simple molecules is a challenging problem[16].

With this end in view, we calculated the energy of interaction of L-L alanine and D-L alanine pair using *ab initio* and semi-empirical levels of theory as a function of distance and orientation between the pair of molecules. The energy surface is explored as follows: (1) keeping individual geometry of optimized alanine molecules as rigid and varying the distance and orientation between them using *ab initio* theory (HF/6-311++G**) (2) by relaxing the selected conformational variables of alanine during rotation using semi-empirical (PM3) level of theory.

Starting from the optimized structures of the non-bonded homochiral (L–L) and heterochiral (D–L) pairs of molecules, the energy surfaces are first studied with rigid geometry by varying the distance and orientation. The intermolecular energy surface of the L–L pair is more favour-

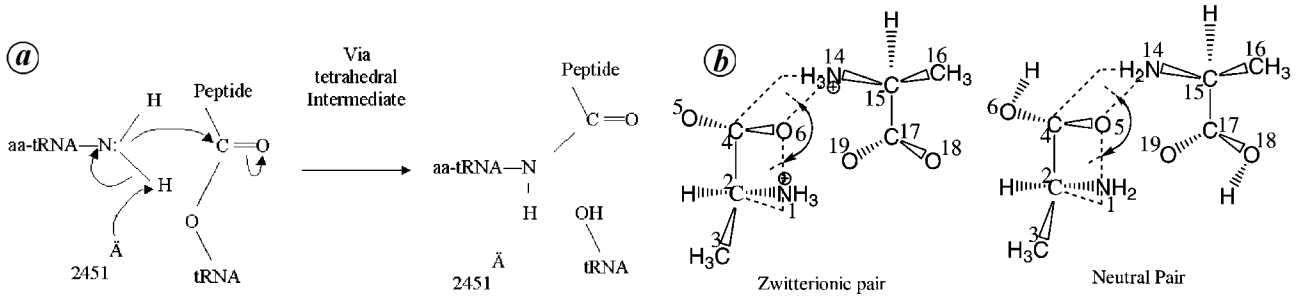

Figure 1. *a*, Peptide biosynthesis reaction scheme. The amino acyl (aa) and peptidyl t-RNA with the amino and carboxylic ends are shown. A2451 is also shown. The intermediate structures are omitted. *b*, Schematic representation of molecular orientations considered in the present work.

able than the corresponding energy surface of the D-L pair. The electrostatic interaction further augments chiral discrimination. The Basis Set Superposition Error (BSSE) corrected results show enhanced discrimination. Use of higher-level theory (MP2) and further BSSE correction do not change the conclusions made at the HF level. The major conclusions based on HF and MP2 level calculations are not altered when the calculations of the potential energy surfaces for neutral and zwitterionic pairs are repeated using DFT (B3LYP/6-311++G**) level of theory[17]. The energy is first optimized for the pair of zwitterionic state of the molecules. Keeping one molecule as reference, the distance between the oxygen atom of the carboxyl group of the reference molecule and the non-bonded N atom of the amino terminal of the other molecule is increased. At each point the N---O′—C′—C$^{\alpha}$ (dotted line indicates that the respective atoms are not covalently bonded) dihedral angle is varied by 2π (note that the charge of the COO^- group is distributed over both oxygen atoms in the zwitterionic structure and the oxygen atom in the N---O′—C′—C$^{\alpha}$ dihedral angle could be denoted as O″)[18]. Justification of such variation is that the proximity of the oxygen atom of the carboxyl group of the reference molecule and non-bonded N atom of the amino terminal of the other molecule is effective in peptide bond formation. As the orientation dependence in the proximity effect of the peptide bond formation is important, variation in the energy of the L–L and D–L pair with the dihedral angle will indicate the chiral discrimination of the corresponding pairs. Explicitly, the 14N---6O—4C—2C dihedral angle for the zwitterionic L–L pair of molecules and the 14N---6O—4C—2C dihedral angle for the zwitterionic D–L pair of molecules are varied for this purpose (Figure 1 *b*). The energy difference between the L–L pair and D–L pair is defined on chiral discrimination energy and is denoted by ΔE_{LL-DL}. The sign of ΔE_{LL-DL} will indicate homo- (when ΔE_{LL-DL} is negative) or heterochiral (when ΔE_{LL-DL} is positive) preference. BSSE is calculated by counterpoise method at few points where maximal chiral discrimination is observed. The aim is to note the extent of BSSE in the energy profile.

The intermolecular energy surface of the alanine pair is further calculated by relaxed geometry optimization using Gaussian 03W suite of programs[19] with semi-empirical PM3 level theory[20]. The intermolecular energy surface is searched systematically by varying the orientation and distance of the alanine pair. First, the conformational space of alanine is explored by a grid search. The following single bonds in alanine are identified for possible dihedral angle variation: Cα–C (COOH), Cα–C (CH3) and Cα–N (NH3). The bond length and bond angles are fixed throughout the calculation. Each of the bonds specified is then rotated through 360° by increments of 30° variation in dihedral angle. Out of the generated conformations, all high-energy conformations are neglected and only low energy conformers are used for the constructing molecular pairs and study of L–L intermolecular energy surface. Similarly, sets of D-enantiomer conformations are also generated by generation of mirror-image structures and are used for the constructing molecular pairs and study of L-D intermolecular energy surface. It may be noted that the lowest energy conformations show that there is no conformational energy difference between L- and D-alanine. The 14N---6O—4C—2C dihedral angle for the L–L and D–L pair of zwitterionic molecules and the 14N---5O—4C—2C dihedral angle for the L–L and D–L pair of neutral molecules are varied and at each point the pair was subjected to optimization at PM3 level (for the relevant angles, see Figure 1 *b*).

The plot of ΔE_{LL-DL} with variation in the N---O—C—C dihedral angle at given distances for rigid geometry of molecules are shown in Figure 2. Variation of distance at given dihedral angles is shown in Figure 3, respectively. The plot shows that the interaction energy of the L–L pair in the zwitterionic state and the corresponding interaction energy of the L–D pair are not identical over the range of orientations considered and exhibit clear discrimination for certain range of mutual orientations. The largest discrimination is observed at the separation at the minimum energy (2.69 Å) and gradually diminishes with increasing separation. Homochiral discrimination (ΔE_{LL-DL} is negative) is observed between the peaks of heterochiral discrimina-

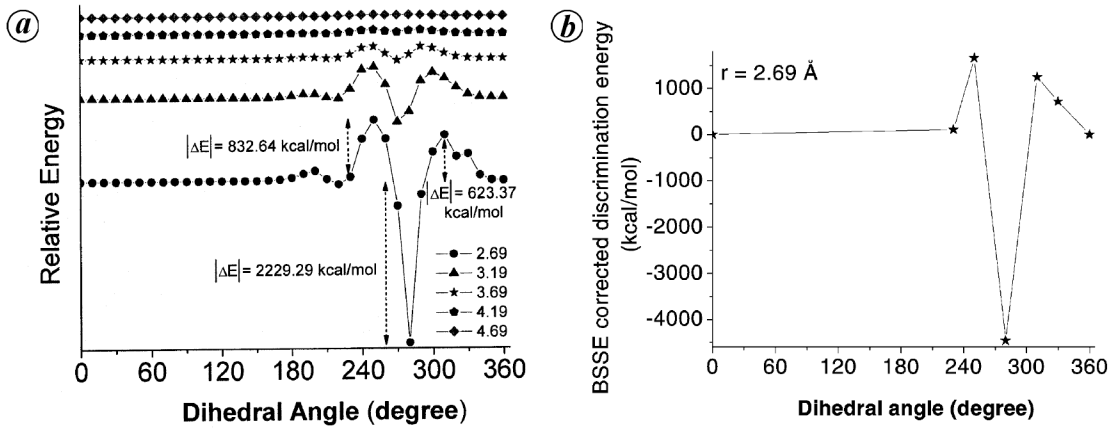

Figure 2. *a*, Plot of ΔE_{LL-DL} with variation in mutual orientation at given distances. ΔE_{LL-DL} is close to zero for all plots at 0° dihedral angle. The scales of all plots are identical and calculations are carried out with rigid geometry. *b*. Plot corresponds to distance at which maximal homochirality and heterochirality are observed in the respective uncorrected energy surfaces. Plot of BSSE corrected energy profile shows chiral discrimination energy[17] ΔE_{LL-DL} values at the HF/6-311++G** level of theory.

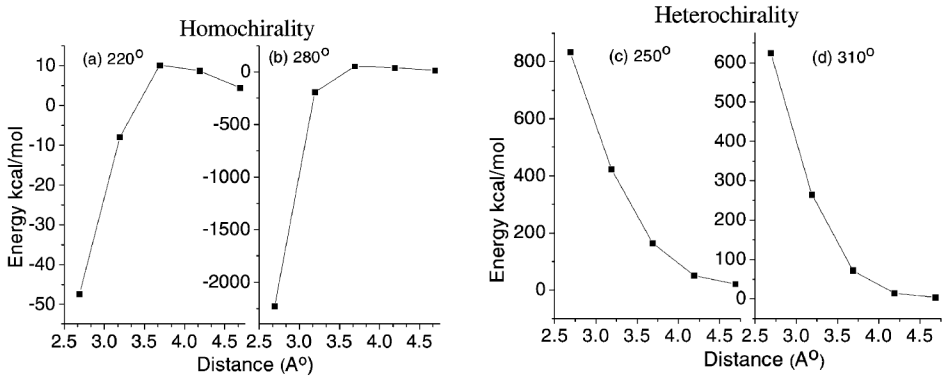

Figure 3. Plot of ΔE_{LL-DL} with variation in separation and at specific orientation values. Calculations are carried out with rigid geometry at the HF/6-311++G** level of theory.

tion. However, the maximal homochirality as measured by the quantity ΔE_{LL-DL} is larger than the maximal degree of heterochirality, measured by same parameter. BSSE correction enhances the chiral discrimination, which is clearly seen in Figure 2 *b*. Hence the observed discrimination in the present work is not significantly dependent on the basis set or BSSE.

The non-monotonous variation of ΔE_{LL-DL} for the rigid geometry can be understood from the variation in the mutual spatial arrangement of atoms surrounding the two chiral centres of the neighbouring alanine molecules and the resulting change in the interaction energy. ΔE_{LL-DL} is negligible at N---O—C—C dihedral angle at minimum energy (defined as 0°). The energy difference of the L–L pair and D–L pair at dihedral angle 280° is –2229.29 kcal/mol. At this point, the L–L pair shows strong electrostatic attraction between the atoms 1-N and 19-O. In addition, very weak short-range interaction is present between the atoms 9-H and 22-H. The D–L pair also shows strong electrostatic attraction between the atoms 6-O and 14-N, as well as strong short-range repulsive interaction between the atoms 6-O and 21-H exits. The D–L pair energy is higher than the L–L pair due to the strong short-range repulsion. Consequently, homochirality is preferred over heterochirality in this orientation regime. The energy difference between the L–L pair and D–L pairs is 832.64 kcal/mol at the dihedral angle value 250° (relative to the dihedral angle at minimum energy defined as 0°). In the L–L pair, the electrostatic repulsion is present between the atoms 1-N and 14-N and also strong short-range repulsion occurs between the atoms 8-H and 14-N, and the L–L pair is higher in energy than the D-L pair. Also strong electrostatic repulsion occurs between the atoms 14-N and 6-N for the D–L pair. Hence heterochirality is preferred over homochirality in this orientation regime. The energy difference of the L–L and D–L pairs of molecules is 623.37 kcal/mol at dihedral angle 310°. Electrostatic attraction occurs between the atoms 19-O and 1-N as also 1-N and 19-O of the L–L pair and D–L pair respectively. In addition, the L–L pair shows strong short-range repulsive interaction between the atoms 7-H and 19-O. Due to additional strong short-range repulsive interaction, the L–L pair energy is higher than that of

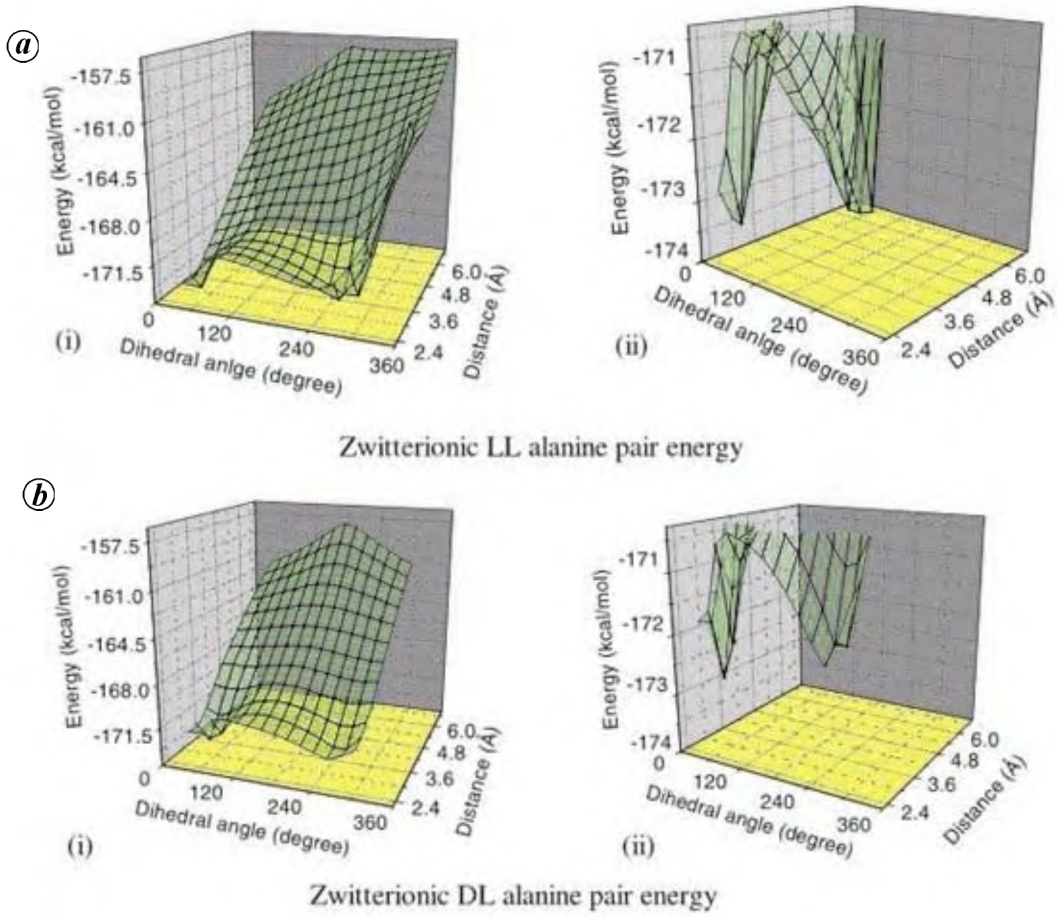

Figure 4. Plot of E_{LL} and E_{DL} with variation in mutual orientation at given distances calculated at PM3 level of theory. Scales for orientation and distance variation of both plots are identical. The energy range of the plot is chosen smaller, in Figure ***a***(ii) and ***b***(ii) to highlight the discrimination. The molecules are allowed to relax the geometry in the zwitterionic state. For details of calculation, see text.

the D–L pair. Thus heterochirality is preferred over homochirality in this orientation.

Similar chiral discrimination features as in the rigid geometry-based calculation are observed when the molecules are allowed to relax. However, the magnitude of the chiral discrimination energy is less in the latter, as compared to the previous case. The energy surface of E_{LL} and E_{DL} with variation in the N---O—C—C dihedral angle at given distances is shown in Figures 4 and 5 for pair energy of zwitterionic and neutral state respectively. At the low energy point, the L–L pair shows strong electrostatic attraction between the atoms 6O---20H-14N and 1N-9H----18O. In addition, weak electrostatic interactions are possible between heteroatom and covalently bonded hydrogen. The D–L pair shows favourable electrostatic interaction between the atoms 6O---20H-14N and 1N-9H----18O. The possible electrostatic interactions are achieved only by different orientation of the molecules. The D–L pair energy is higher than the L–L pair energy due to the spatial arrangement of the ancillary methyl group attached in the chiral carbon. In the case of D–L pair, methyl groups are sterically hindered to the reacting group but in L–L pair methyl groups are far from the reacting group. Consequently, homochirality is preferred over heterochirality in this orientation regime. In the case of neutral alanine pair interaction, the energy difference between the E_{LL} and E_{DL} is less than the zwitterionic state. This is because the electrostatic interaction between the neutral alanine pair is far less than the zwitterionic pair. It is also expected that as the pair of alanine molecules is allowed to relax its geometry, the effect of chirality is averaged out significantly.

The observed orientation dependence is relevant in peptide biosynthesis where the proximity effect is indicated as important. If the interaction energy profile for the rotational motion of A to P site is identical as well as independent of orientation of the dihedral angle, then it is expected that there would be no energetic advantage of L–L synthesis over D–L synthesis. However, the present calculation is based on zwitterionic and neutral state of alanine, which is different from the ionization states of the amino acids at the A and P sites. Hence, at present we

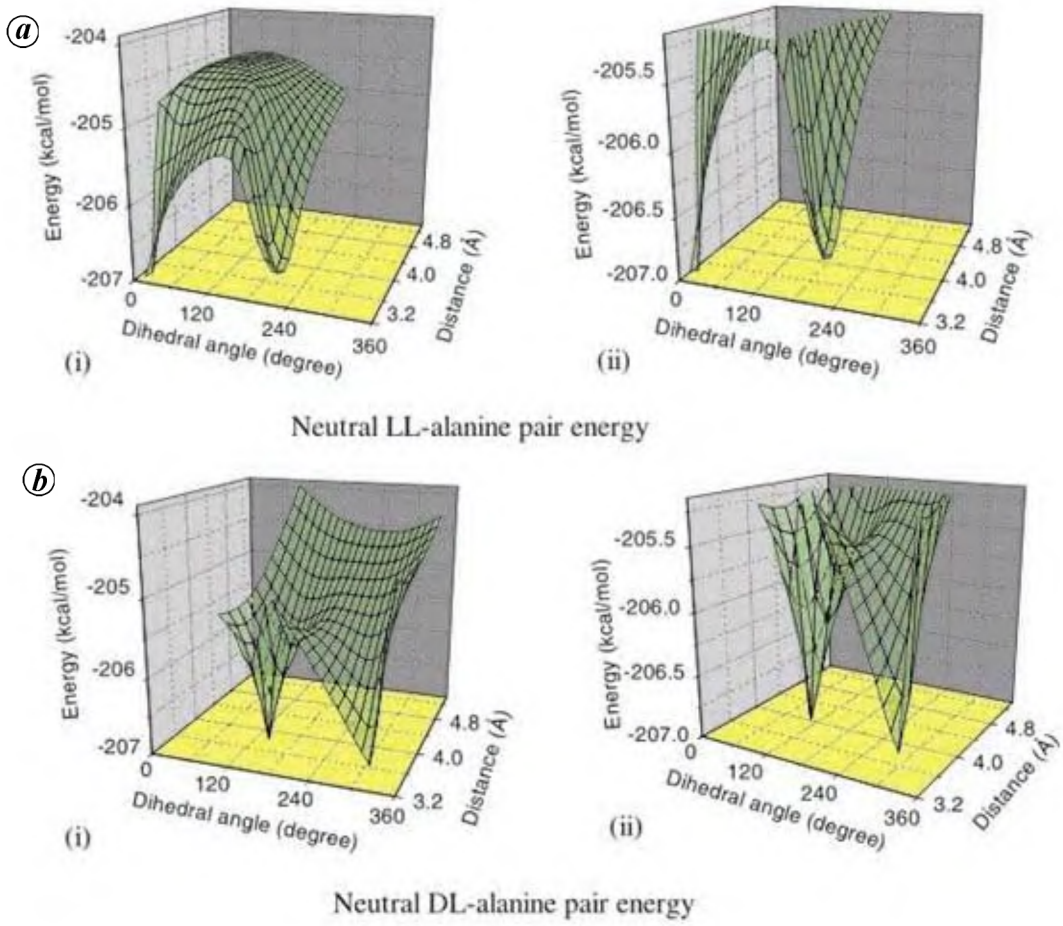

Figure 5. Plot of E_{LL} and E_{DL} with variation in mutual orientation at given distances calculated at PM3 level of theory. Scales for orientation and distance variation of both plots are identical. The molecules are allowed to relax the geometry in the neutral state. The energy range of the plot is chosen smaller in Figure *a*(ii) and *b*(ii) to highlight the discrimination. For details of calculation, see text.

can only conclude that homochirality is preferred over heterochirality in the case of both zwitterionic and neutral states. A more detailed study of relaxed geometry optimization with higher-level *ab initio* theory is under way.

In summary, we studied chiral discrimination of alanine molecule using *ab initio* theory (HF/6-311++G**) with rigid geometry and also by semi-empirical level of theory (PM3) where the molecules are allowed to relax their geometry and can deviate from their isolated optimized state for both homochiral pair (L–L) and heterochiral pair (D–L). The pair energy is calculated with rigid geometry and also relaxed geometry optimization by varying the distance and orientation between molecules. The study reveals clear discrimination and homochirality is preferred over heterochirality in both cases. Nonmonotonous distance and orientation-dependence is observed. It is known that proximity and orientation play a significant role in peptide biosynthesis. The present study shows that the exclusive incorporation of L-amino acid by nature over D-amino acid could possibly be related with the observed preferred homochirality at specific orientations.

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Effect of ectomycorrhizal fungal species on the competitive outcome of two major forest species

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Mycorrhizae-mediated processes are known to influence the growth performances of host species in plant communities, but not much is known about their role in competitive outcome of host species. We show that the outcome of competition between the seedlings of two major Indian Himalayan tree species, viz. ban oak (*Quercus leucotrichophora*) and chir pine (*Pinus roxburghii*) is changed with the change in ectomycorrhizal fungal species. While oak does better than pine when grown in a mixed culture in the presence of *Russula vesca*, the outcome is reversed in the presence of *Amanita hemibapha*.

Keywords: Biodiversity, biofertilisers, ecosystem, ectomycorrhizae, Himalaya, productivity.

MYCORRHIZAE are known to influence plant performance through the benefits they confer on their hosts. Their benefits, for example, lead to improved growth of host plants and increased tolerance to drought and disease[1]. Mycorrhiza-mediated processes are likely to influence plant nutrition, plant competition and soil nutrient cycling[2]. More than 90% of plant species have association with mycorrhizal fungi[3], but not much is known about the effects of mycorrhizal symbiosis on plant species composition and competition[4]. The importance of mycorrhizal fungi in determining plant diversity relative to other mechanisms such as species competition and species coexistence has been little studied. The role of mycorrhizal fungi in nutrient uptake by host plant may vary from one group of fungi to another, and with changing environmental condition. Through a conceptual model, Aerts[5] schematically showed that the type of mycorrhizal association, such as ericoid mycorrhizal fungi and arbuscular mycorrhizal fungi, determines the plant species which dominate in heathland ecosystem. It is likely that the mycorrhizal effect between species, on the nutrient uptake of the host plant also varies from one species to another within the same group of mycorrhizal association. Different species of ectomycorrhizal fungi differ in their responses to host plants[6,7]. Colonization of mycorrhizal fungi is reported to reduce competitive dominance between host species and promote species diversity[8,9], so as to increase competition between them[10].

The main objective of the present study is to examine whether competitive outcome of the tree species occur-

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