# Structural analyses of $\beta$-amino acid containing peptides

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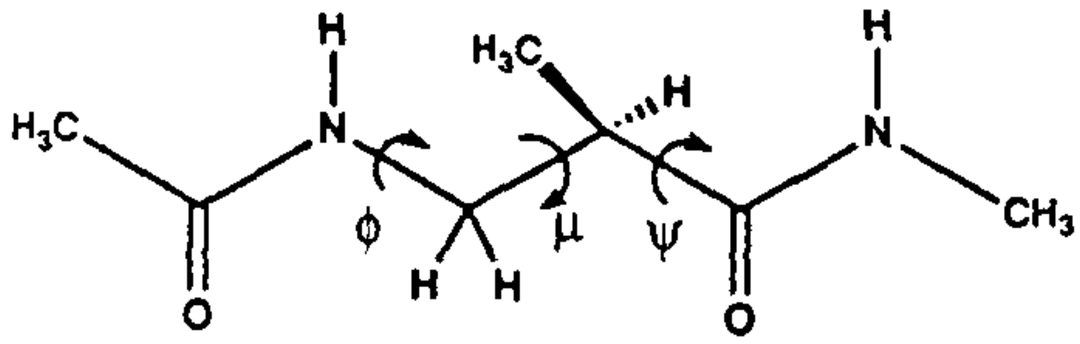
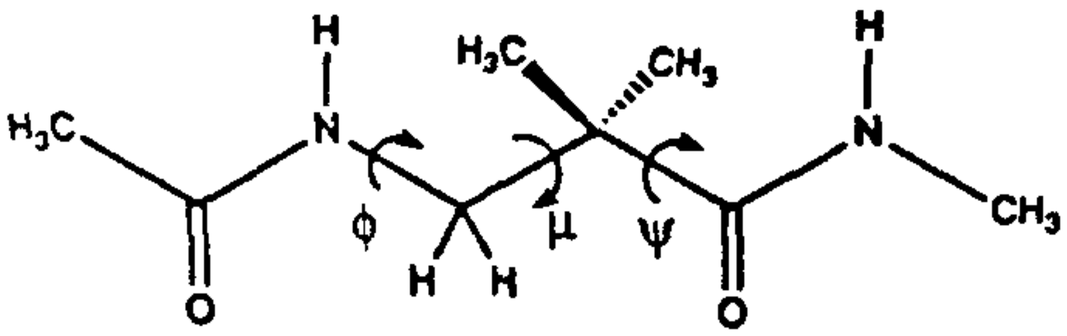
**Unnatural amino acids (such as $\beta$-amino acids) are useful research tools in peptide chemistry. We present conformational energy calculations using molecular mechanics (MM2) on two model compounds containing $\beta$-amino acid derivatives. The low energy models are characterized by a lack of intramolecular hydrogen bonding interactions, qualitatively consistent with the results of the IR studies. X-ray crystal structures of $\beta$-amino acid-containing compounds lie within 3 kcal/mole of the global minimum model. Stereochemical guidelines for the incorporation of $\beta$-peptide residues have been proposed.**

PEPTIDES are one of the most widely studied class of compounds in the course of drug discovery. Endogenous peptides, with well-characterized physiological receptors, have been recognized as vital biological effectors such as hormones and neurotransmitters[1]. As peptide-based drugs suffer from the disadvantage of poor oral *bioavailability*[2], *a variety of* strategies to design peptidomimetics has evolved to provide enhanced metabolic stability without any loss of biological activity[3-6]. Somatostatin and LHRH analogs exemplify such strategies[3,7].




**1**




**2**

**Figure 1.** Schematic representation of model compounds **1** and **2** and the definition of the three conformational parameters, $\varphi$, $\mu$ and $\psi$. The three torsions are defined along the 'backbone' of the pseudopeptides **1** and **2** as [$\varphi$(C–N–C$^{\alpha}$–C$^{\beta}$); $\mu$(N–C$^{\alpha}$–C$^{\beta}$–C); $\psi$ (C$^{\alpha}$–C$^{\beta}$–C–N)].

Here, we present modifications to peptide structures by replacing an $\alpha$-amino acid by a $\beta$-amino acid derivative ($\alpha$-methyl-$\beta$-alanine; AMBA) **1** and **2** (Figure 1). Crystallographic data on $\beta$-peptides are limited[8-14]. IR studies have provided some insights into intramolecular hydrogen bonding preferences in $\beta$-amino acid-containing compounds[8,15]. We have used the methods of molecular mechanics to understand the energetic preferences of various conformations of **1** and **2**. Our results are qualitatively consistent with the crystallographic and IR observations.

The pseudopeptides **1** and **2** were model-built in an extended conformation using MacroModel (v.3.0) (ref. 16) and energy-minimized using MM2[17] at a dielectric constant of 4.0. The minimized models were then used to generate conformations as a function of $\varphi$, $\mu$ and $\psi$ varied at 30° intervals from 0° to 360° and optimized in two stages. First, $\varphi$, $\mu$ and $\psi$ were constrained to their starting values with a harmonic force constant of 1000 kcal/mole°, while the rest of the structures were allowed to energy optimize in cartesian space. In the second stage, the output of the first stage was optimized by allowing all degrees of freedom to move. The resultant sets of energy minima for **1** and **2**, are designated as S1 and S2, respectively.

The collections of unique structures for **1** and **2** were analysed for the distributions of $\varphi$, $\mu$ and $\psi$. Two of these with most widespread distributions were then chosen to carry out further conformational energy calculations by their systematic variations at 10° intervals, ranging from 0° to 360°. During such calculations, the two torsions were constrained as above while no constraints were placed on the third angle. The conformational energy calculations on **1** were carried as a function of $\varphi$ and $\mu$, while the corresponding calculations for **2** were done as a function of $\mu$ and $\psi$, for reasons stated in the later text (*vide supra*).

All the energy calculations were done on the Silicon Graphics 4D/240 GT at Searle consuming a total of about 12 hours of CPU time for both the molecules. The processing of data was done through the program CONMAP (written by one of us, VNB) which took about 3 minutes on a VAX8650.

Set S1 has eleven structures (Table 1), which are all within 1 kcal/mole of the global minimum (Figure 2). The torsion $\psi$ has only two sets of values, one centered around $-60°$ and the other around 180°. The torsion $\varphi$ also has only two sets of values, but their ranges are larger than that of $\psi$. $\mu$ varies over the three standard ranges centered around $\pm 60°$ and 180°. Hence, conformational energy calculations on **1** were carried out as a function of $\varphi$ and $\mu$ while keeping $\psi$ around $-60°$ and 180°. Tables 3 and 4 list the energy minima in these calculations starting with the global minima.

**Table 1.** Conformational parameters $\varphi$, $\mu$ and $\psi$ (Figure 1) and energies relative to global minimum (M1) in energy refined models of **1**

| Model | $\varphi$ | $\mu$ | $\psi$ | Relative energy |
|---|---|---|---|---|
| M1 | 106 | −57 | −63 | 0.00 |
| M2 | −111 | −62 | −171 | 0.02 |
| M3 | 102 | 56 | −170 | 0.13 |
| M4 | −111 | −59 | −66 | 0.36 |
| M5 | 112 | 172 | −170 | 0.56 |
| M6 | −108 | 63 | −77 | 0.56 |
| M7 | 90 | 55 | −79 | 0.68 |
| M8 | −91 | 175 | −68 | 0.73 |
| M9 | −92 | 176 | −168 | 0.76 |
| M10 | 114 | 174 | −67 | 0.81 |
| M11 | −133 | 60 | −169 | 0.93 |

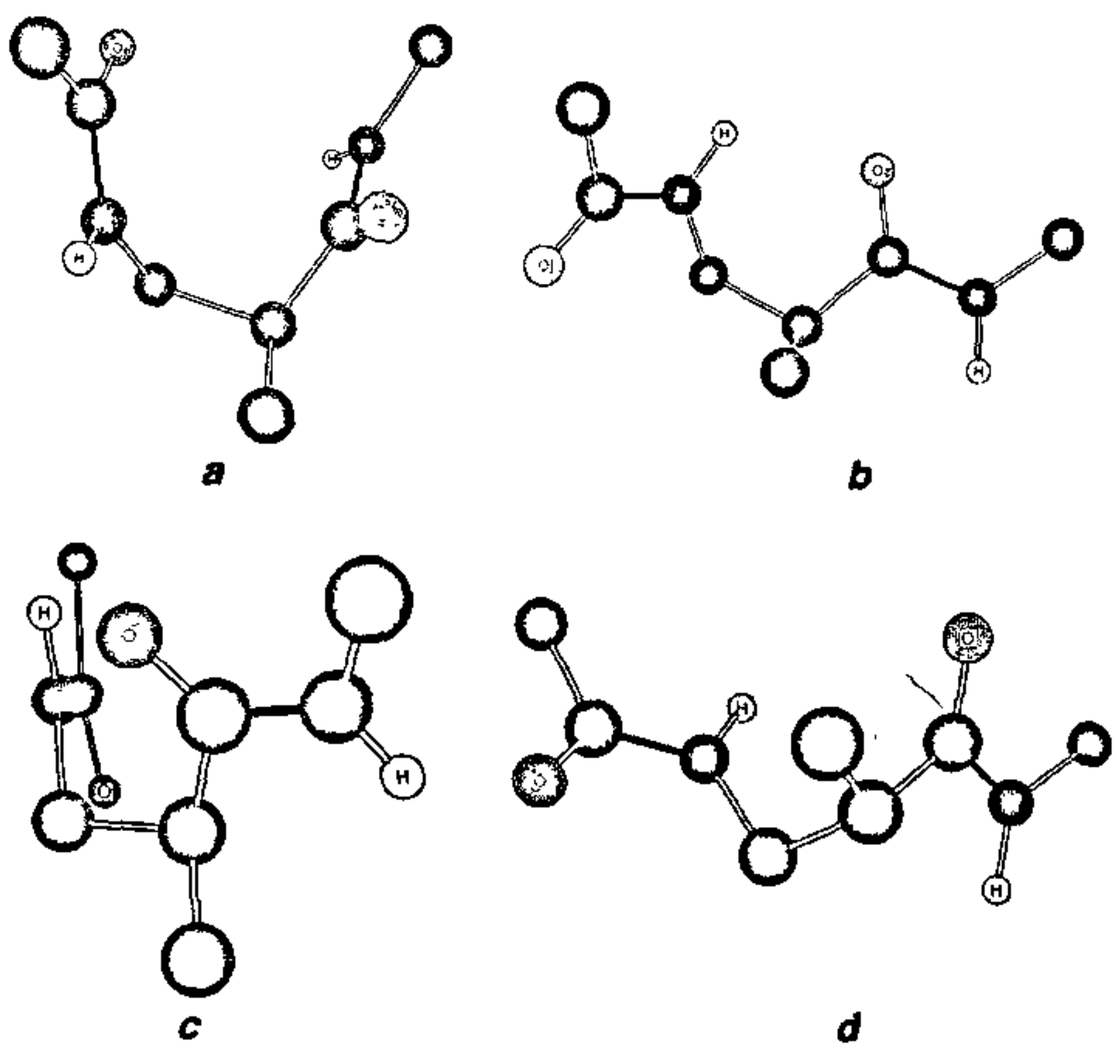



**Figure 2.** Computer graphics illustrations of four low-energy minima M1−**1**(*a*), M2−**1**(*b*) and M3−**1**(*c*) and M4−**1**(*d*) listed in Table 1. Protons on carbons have been omitted for clarity.

Set S2 has 9 structures (Table 2), all of which are within 2 kcal/mole of the global minimum (Figure 3). $\varphi$ is around −110° and 110° while $\mu$ and $\psi$ vary around ±60° and 180°. In this light, conformational energy calculations on **2** were carried out as a function of $\mu$ and $\psi$ while keeping $\varphi$ around −110° and 110°. Tables 5 and 6 list the energy minima starting with the global minimum, in these calculations.

**Table 2.** Conformational parameters $\varphi$, $\mu$ and $\psi$ and energies relative to global minimum (M1) in energy refined models of **2**

| Model | $\varphi$ | $\mu$ | $\psi$ | Relative energy |
|---|---|---|---|---|
| M1 | −109 | 53 | 59 | 0.00 |
| M2 | −111 | −59 | 179 | 0.11 |
| M3 | 111 | 55 | 61 | 0.46 |
| M4 | 104 | 178 | 64 | 0.83 |
| M5 | −107 | −179 | −179 | 0.97 |
| M6 | −109 | 179 | 63 | 1.05 |
| M7 | −108 | 64 | −73 | 1.08 |
| M8 | 102 | 57 | −76 | 1.24 |
| M9 | 130 | −62 | 178 | 1.56 |

The minima in Table 3 have a mirror symmetry about $(\varphi,\mu)=(0°,0°)$. The transitions to barriers between various minima is significantly higher (~ 6 kcal/mole) *via* the $\varphi=0°$ pathway than *via* the $\varphi=180°$ pathway (~1–3 kcal/mole). The barrier to transition between M1 and M2 in Table 4 is around 6 kcal/mole *via* the $\varphi=0°$ and $\varphi=180°$ pathways, while it is greater than 10 kcal/mole for any path involving the $\varphi=0°$ axis.

**Table 3.** Energy minima of **1** in the conformational space $(\varphi,\mu)$ at $\psi\sim-60°$. The global minimum (minimum #1) energy is 0.32 kcal/mole

| Minimum number | $\varphi$ | $\mu$ | Relative energy |
|---|---|---|---|
| 1 | 100 | −60 | 0.00 |
| 2 | −110 | −60 | 0.37 |
| 3 | −110 | 60 | 0.53 |
| 4 | 90 | 60 | 0.67 |
| 5 | −90 | 170 | 0.72 |
| 6 | 110 | 170 | 0.80 |

**Table 4.** Energy minima of **1** in the conformational space $(\varphi,\mu)$ at $\psi\sim180°$. The global minimum (#1) energy is 0.3 kcal/mole

| Minimum number | $\varphi$ | $\mu$ | Relative energy |
|---|---|---|---|
| 1 | −110 | −60 | 0.00 |
| 2 | 100 | 60 | 0.15 |
| 3 | 110 | 170 | 0.54 |
| 4 | −90 | −180 | 0.78 |
| 5 | −130 | 60 | 0.90 |
| 6 | −90 | 60 | 0.99 |
| 7 | 90 | −40 | 1.24 |
| 8 | 130 | −60 | 1.36 |
| 9 | 80 | −80 | 2.59 |

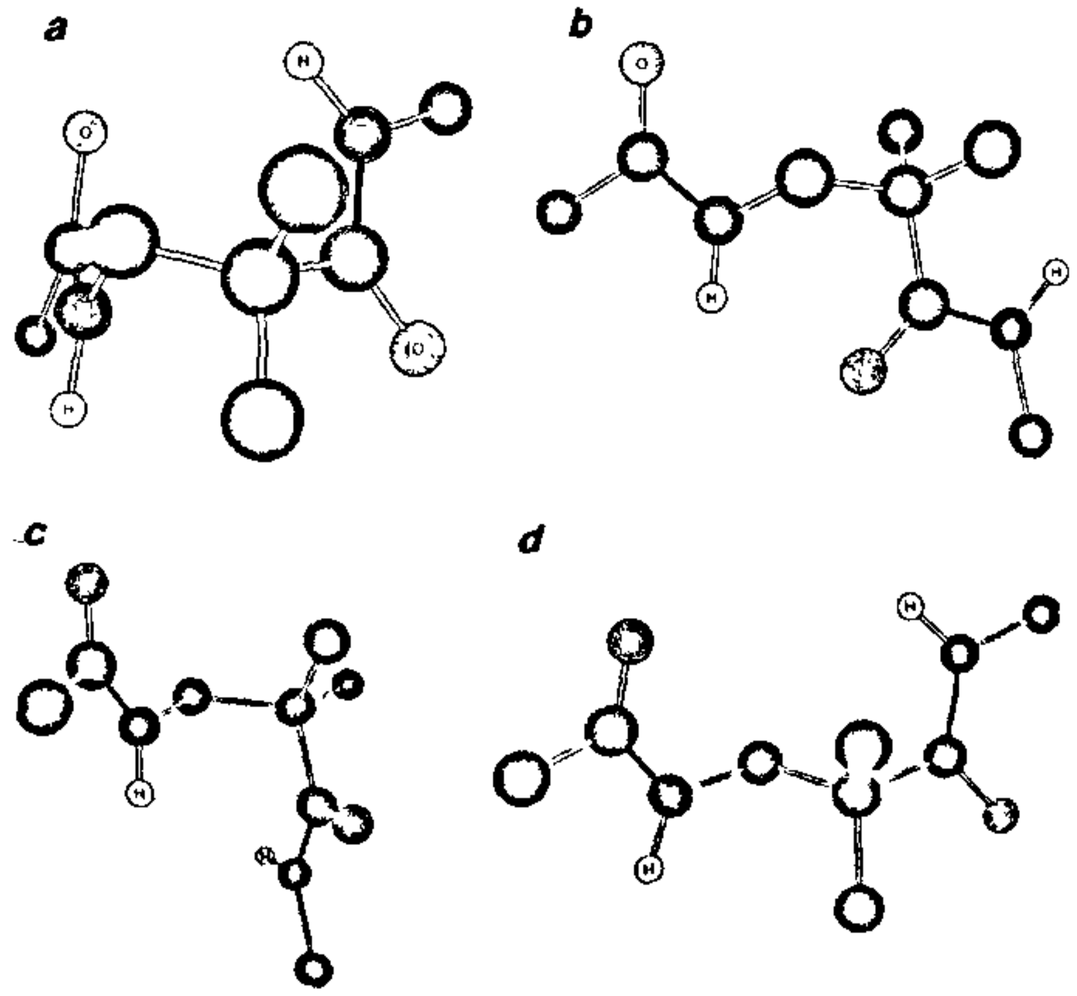



**Figure 3.** Computer graphics illustrations of four low energy minima M1-**2**(*a*), M2-**2**(*b*), M3-**2**(*c*), and M4-**2**(*d*), listed in Table 2. Protons on carbons have been omitted for clarity.

**Table 5.** Energy minima of **2** in the conformational space $(\mu,\psi)$ at $\varphi \sim 110$. The global minimum (# 1) energy is 2.32 kcal/mole

| Minimum number | $\mu$ | $\psi$ | Relative energy |
|---|---|---|---|
| 1 | −50 | −60 | 0 00 |
| 2 | 60 | −180 | 0.10 |
| 3 | 50 | 60 | 0 50 |
| 4 | 180 | 60 | 0 87 |
| 5 | 180 | 180 | 0.96 |
| 6 | 180 | −60 | 1.05 |
| 7 | −60 | 70 | 1.12 |
| 8 | 60 | −80 | 1.26 |
| 9 | −60 | 180 | 1.55 |

**Table 6.** Energy minima of **2** in the conformational space $(\mu,\psi)$ at $\varphi \sim -110$. The global minimum (minimum #1) energy is 2.35 kcal/mole

| Minimum number | $\mu$ | $\psi$ | Relative energy |
|---|---|---|---|
| 1 | 50 | 60 | 0.00 |
| 2 | −60 | 180 | 0.06 |
| 3 | −50 | −60 | 0 47 |
| 4 | 180 | −60 | 0.82 |
| 5 | 180 | 180 | 0.93 |
| 6 | 180 | 60 | 1.01 |
| 7 | 60 | −70 | 1.09 |
| 8 | −60 | 80 | 1.23 |
| 9 | 60 | 180 | 1.52 |
| 10 | −70 | 20 | 3.82 |

In the $(\mu,\psi)$ space of **2**, for $\varphi = 110°$, M4 has an extended structure with *trans* configuration about the C1–$C^{\alpha}$ and $C^{\alpha}$–C1 bonds. The barriers to transitions between various minima are around 2 to 4 kcal/mole *via* most pathways. Nine symmetrically disposed regions have energy values greater than 8 kcal/mole. The global minima in the two tables (5 and 6) have practically identical energy values (2.32 kcal/mole and 2.35 kcal/mole, respectively). Similar percentages (62.4 for $\varphi = 110°$ and 62.9 in $\varphi = -110°$) of the total conformational space are enclosed within the 5 kcal/mole limit.

Comparison of the sets of minima for **1** and **2** bring forth the following points. The global minima of **1** and **2** occur with a $(\varphi, \mu, \psi)$ combination of (−110, −60, 180) and −110, 50, 60), respectively, consistent with the results in Tables 1 and 2. The former structure is more extended than the latter. A compact structure of **1** with conformations similar to M1−**2** has an energy of 0.37 kcal/mole above the global minimum. On the other hand, an extended structure of **2** with conformations similar to M1-**1** has an energy of 0.06 kcal/mole above the global minimum. Thus, both the combinations of $(\varphi, \mu, \psi)$ can be adopted by either **1** or **2**.

The energy refined models of **1** and **2** are characterized by the lack of intramolecular hydrogen bonding interactions. Only M2 of both the molecules has the N-terminal N-H hydrogen located at about 2.3 Å from the C-terminal C=O oxygen. The corresponding distance is larger in rest of the energy minima. This observation is qualitatively consistent with the destabilizing effect of $\beta$-alanine as seen from comparative IR studies[8,15] on tBu-CO-L-Pro-Gly-NHMe and tBu-CO-L-Pro-$\beta$-Ala-NHMe.

Based on the energy refined models, we propose the following conformational guidelines for molecules with $\beta$-Ala residues. The torsion $\varphi$ can adopt values of around ±110°, while $\psi$ can adopt values of either −60° or 180°. The torsion $\mu$ can adopt any of three standard staggered conformations. We have compared our results with the X-ray crystal structures of a few compounds containing $\beta$-alanine[8-14]. The conformations of $\beta$-alanine in the structures of two cyclic peptides are qualitatively similar to M2 of **1** which is very close in energy to the global minimum (Table 1). The two structures are characterized by $(\varphi, \mu, \psi)$ values of (−103°, −58°, 169°) and (−137°, −77°, 167°). In a modified Pro-$\beta$-Ala dipeptide, the crystal conformation $[(\varphi,\mu,\psi) = (87°, -159°, 180°)]$ is qualitatively close to M5 in **1** which is destabilized relative to its global minimum by ~0.6 kcal/mole. A modified $\beta$-Ala-His dipeptide with protonated N-terminus has a $(\mu,\psi)$ combination of (183°, 141°). This is destabilized by about 1.5 kcal/mole relative to the global minimum in the $(\mu,\psi)$ space for $\varphi = 110°$ and by about 3 kcal/mole in the $(\mu,\psi)$ plot for $\varphi = -110°$. Two cyclic peptides containing $\beta$-alanine[10,13] have conformations which are not found as unique energy minima in our analyses. For example, the crystal structures have some values of $\varphi \sim 170°$ and $\psi \sim 100°$. However, these conformations have an energy of less than 3 kcal/mole relative to the global minima for **1** and **2**.

The above comparisons indicate that our calculations find crystal conformations to lie within a reasonable energy range relative to the global minima of **1** and **2**. The qualitative closeness between the crystallographic data and the results of our calculations is encouraging in light of the fact that our calculations have been done (a) without the inclusion of crystal packing forces and (b) with a simplistic treatment of electrostatic effects.

Conformational studies on two model compounds with $\beta$-alanine derivatives indicate that despite the larger size of the unnatural amino acid, the allowed degrees of conformational freedom are surprisingly restricted. Two of the torsions can adopt only two narrow ranges of values, while the third has larger freedom. The energy refined models are qualitatively consistent with published IR studies on peptides with $\beta$-alanine. The backbone conformations of this residue as found in crystal structure studies are within 3 kcal/mole of the global minimum structures predicted from the present investigations. Conformational guidelines have been outlined for the design of compounds containing $\beta$-amino acids.

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# Studies on DNA modification in *Oscillatoria* sp. MKU 178

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**Cyanobacterial DNA are generally believed to be resistant to restriction by endonucleases due to extensive modification of the DNA. We report here the absence of a *dam*-mediated modification in a cyanobacterium, *Oscillatoria* sp. MKU 178.**

RESTRICTION and modification systems of DNA have been well documented in several organisms[1] and the roles which have been postulated for the same include, protection of cells against invasion by foreign DNA[2], replication and repair of DNA and control of gene expression[3,4]. However, as the type of restriction/modification is distinct for each organism, the worker interested in using an organism for recombinant DNA work, needs a thorough understanding of its restriction/modification system to be successful in his venture. Our laboratory has been working on the genetic and physiological aspects of cyanobacteria and our present study is aimed at studying the DNA modification in cyanobacteria. We took for our study, *Oscillatoria* sp., a non-heterocystous cyanobacterium, which exists in a wide range of habitats like streams, ponds, lakes, irrigation canals, rice fields and even sewage and industrial wastewaters[5]; besides, it is useful to agricultural lands because of its soil-binding property and polysaccharide production. These properties make it an ideal choice for the introduction and expression of other agronomically important traits such as production of plant growth regulators and insecticides for its better use in agriculture.

Earlier workers have reported that DNA from several cyanobacterial species is resistant to cleavage by endonucleases[6,7]. In the course of our investigation on the resistance of cyanobacterial DNA to hydrolysis by restriction enzymes we identified one isolate, *Oscillatoria* sp. MKU 178, which was susceptible to cleavage by *Eco*R1. We took up a more detailed study in this isolate.

A fifteen-day-old culture of the strain, grown under a light/dark cycle of 16 h/8 h, at 25° C, was harvested for DNA extraction. DNA was purified following Sambrook *et al.*[8] The DNA was treated with *Dpn*1, *Mbo*1 and *Sau*3A enzymes (New England Biolabs, USA). The tubes were incubated at 37° C for 1 h and then electrophoresed on a 0.7% agarose gel. The DNA was visualized on a UV transilluminator. As can be seen from Figure 1, *Dpn*1 failed to cut the DNA (lane 3), while *Mbo*1 produced a streak showing partial digestion (lane 5) and *Sau*3A totally digested the DNA (lane 7). The corresponding controls showed no digestion. The above three enzymes are isoschizomers recognizing the sequence GATC. However the ability to cut depends on the nature of DNA methylation. The action of *Dpn*1 and *Mbo*1 is mutually exclusive because *Dpn*1 cuts the DNA if the adenine residues are