Theoretical Studies on Peptidoglycans. I. Effect of L-Alanyl, D-Butyl, or D-Valyl Residues at the Positions 4 or 5 of the Pentapeptide Moiety of Peptidoglycan on the Cross-Linking Reaction*

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Synopsis

Possible conformations of X-D-alanyl[4]-D-alanine[5] and its analogs X-L-analyl[4]-D-alanine[5], X-D-alanyl[4]-L-alanine[5], X-D-butyl[4]-D-alanine[5], X-D-alanyl[4]-D-butyric acid[5], X-D-valyl[4]-D-alanine[5], and X-D-alanyl[4]-D-valine[5] have been analyzed by theoretical methods. These studies suggest that L-alanine and D-valine at the 4 or 5 position of the pentapeptide moiety of peptidoglycan will drastically reduce the cross-linking in peptidoglycan biosynthesis, whereas the effect of D-butyric acid will be marked at the 4 position and moderate at the 5 position. This is in good agreement with experimental results. The cross-linking enzyme transpeptidase requires a specific conformation for the 4th and 5th residues for optimal binding.

INTRODUCTION

The peptidoglycan layer in most bacterial cell walls is essential to preserve the shape and rigidity of the bacterium. This layer is a complex network of linear glycans and short peptides. The glycan strand is made up of the repeating unit —(GluNac—β(1-4)-MurNac)—, where *N*-acetylglucosamine is abbreviated as GluNac and *N*-acetylmuramic acid as MurNac, and the short peptide has the general sequence —L-Ala[1]-D-Glu[2]-L-R[3]-D-Ala.[4]. The tetrapeptide is attached to the glycan strand by an amide linkage to the muramic acid residue. Normally, the fourth D-Ala from one glycan strand is cross-linked to the side chain of the third amino acid residue (sometimes the second residue) of the neighboring strand, either directly or through a few amino acid residues to form the netlike structure. The cross-linking or bridging reaction is carried out by the enzyme peptidoglycan transpeptidase. Before this reaction takes place the peptide linked to muramic acid is a pentapeptide, the fifth residue being another D-alanine, which is released during the cross-linking reaction. It is well established by various workers[1,2] that penicillin blocks this reaction by irreversibly binding with the enzyme transpeptidase.

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Chemical analysis of peptidoglycan from various bacteria reveals[2–5] that the bridging pattern and the nature of the bridge amino acid residues vary from bacteria to bacteria. The L-R[3] residue is also different in different bacteria. For instance, in *Micrococcus luteus* it is L-lysine and in *E. coli* it is diaminopimelic acid. Minor variation at the first residue is also observed. But the fourth and fifth residue is always D-alanyl[4]-D-alanine[5]. Configurational change or substitution by other amino acids[6,7] at the 4 or 5 position reduces the cross-linking reaction to varying degrees, indicating the requirement of D-alanine at these positions.

Recently, we have shown[8] from stereochemical criteria that penicillin and other β-lactam antibiotics can assume conformations similar to X-D-alanyl[4]-D-alanine[5]. Because of this conformational similarity, these antibiotics block the cross-linking reaction by irreversibly binding at the active site of transpeptidase, which should bind with the fourth and fifth residues of the pentapeptide moiety. The conformation of the antibiotic or that of the peptide segment is shown to be critical for binding with the enzyme. In the light of these results, the effect of configurational change or substitution by D-butyl (α-amino-*n*-butyl) or D-valyl residues at the 4 or 5 position of the pentapeptide on the conformation of the terminal dipeptide segment has been investigated and the results are presented here. Such a study provides a stereochemical explanation for the effect of L-alanine, D-butyric acid (α-amino-*n*-butyric acid), and D-valine at the 4 or 5 position of the pentapeptide on the cross-linking reaction in peptidoglycan biosynthesis.

PROCEDURE

Conventions

The atoms of the pentapeptide moiety of the peptidoglycan are numbered and the dihedral angles defined according to the IUPAC-IUB Convention.[9] Only the terminal dipeptide segment X-D-alanyl[4]-D-alanine[5] and four of its analogs are shown in Fig. 1. X represents the remaining portion of the pentapeptide. The coordinates of all these dipeptide segments were generated using standard bond lengths and bond angles.[10] The peptide group was kept in the transplanar conformation and the hydrogen atoms of the methyl groups were fixed in the staggered conformation. The rotational angles (ϕ_4, ψ_4) and (ϕ_5, ψ_5) shown in Fig. 1 correspond to (ϕ_2, ψ_2) and (ϕ_3, ψ_3) of our earlier paper.[8]

Steric Maps of D-Butyl and D-Valyl Residues, and C-Terminal L- and D-Alanine

Butyl or valyl residues differ from the alanyl residue by having one or two methyl groups in the place of a hydrogen atom (Fig. 1). In order to gain a qualitative understanding about the effect of such bulky side groups on

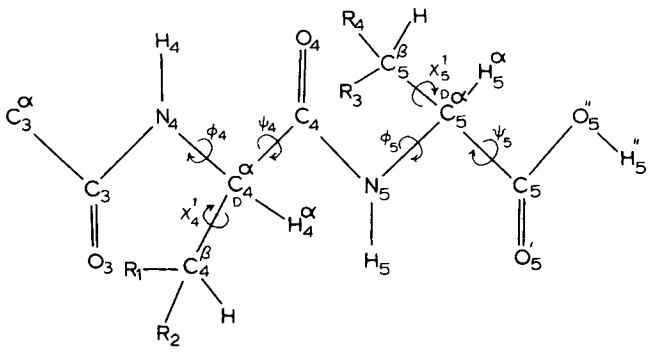

Fig. 1. Schematic representation of X-D-alanyl[4]-D-alanine[5] ($R_1 = R_2 = R_3 = R_4 = H$); X-D-butyl[4]-D-alanine[5] ($R_1 = CH_3$, $R_2 = R_3 = R_4 = H$); X-D-alanyl[4]-D-butyric acid[5] ($R_1 = R_2 = H$, $R_3 = CH_3$, $R_4 = H$); X-D-valyl[4]-D-alanine[5] ($R_1 = R_2 = CH_3$, $R_3 = R_4 = H$); X-D-alanyl[4]-D-valine[5] ($R_1 = R_2 = H$, $R_3 = R_4 = CH_3$).

the backbone dihedral angles (ϕ_4, ψ_4), dipeptide maps with butyl or valyl side chains were constructed by the method of contact criteria.[11] The maps corresponding to the three staggered side-chain orientations were superposed over one another and are shown in Figs. 2 and 3. The (ϕ_5, ψ_5) steric map of C-terminal L- and D-alanine, constructed in the same manner, is shown in Fig. 4.

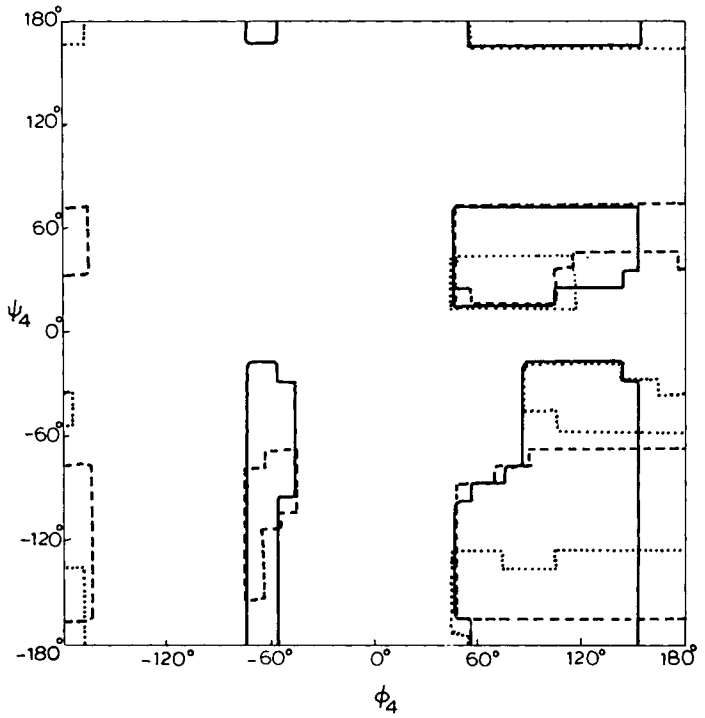

Fig. 2. Steric map for the D-butyl residue for different side-chain orientations: —, $\chi_4^1 = 60°$; - - - -, $\chi_4^1 = 180°$; · · · ·, $\chi_4^1 = -60°$.

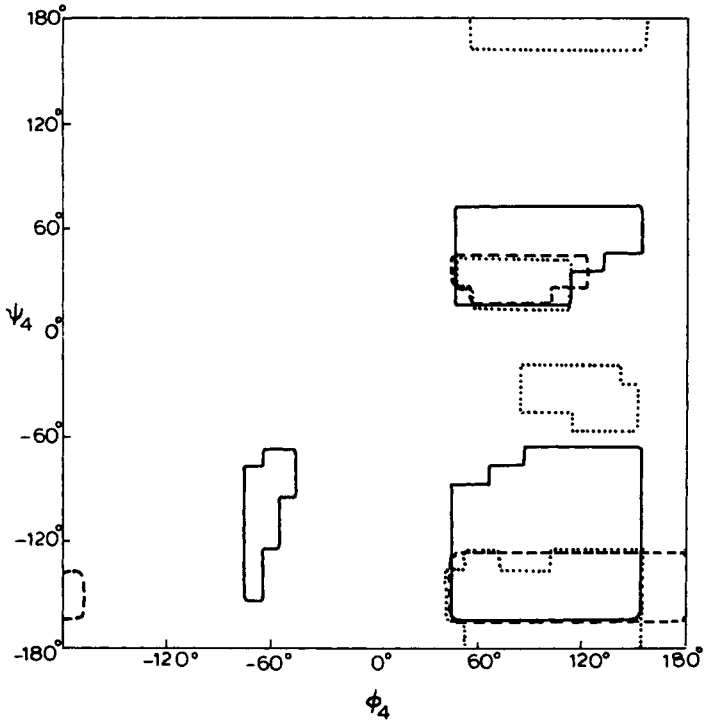

Fig. 3. Steric map for the D-valyl residue for different side-chain orientations: —, $\chi_4^1 = 60°$; - - - -, $\chi_4^1 = 180°$; · · · ·, $\chi_4^1 = -60°$.

Potential-Energy Functions

The following functions were used to calculate the total potential energy (nonbonded + electrostatic) of the dipeptide segments:

$$V_{tot} = V_{nb} + V_{es} \tag{1}$$

$$V_{nb} = F\epsilon(r_0/r_{ij})^{12} - 2.0\epsilon(r_0/r_{ij})^6 \tag{2}$$

$$V_{es} = 166\frac{q_i q_j}{r_{ij}} \tag{3}$$

V_{tot} is the total potential energy of the molecule; V_{nb} and V_{es} are the nonbonded and electrostatic contributions in kcal/mol, respectively; r_{ij} is the interatomic distance in angstroms; ϵ is the depth of the nonbonded energy minimum; and r_0 is the interatomic distance at the minimum. F has the value of 0.5 for 1–4 interactions or 1.0 for 1–5 and higher interactions; and q_i, q_j are the partial atomic charges. The constants used are those reported by Momany et al.[12] and the charges are those of Poland and Scheraga.[13] No torsional barriers were considered for ϕ, ψ rotations for reasons cited by Momany et al.[14]

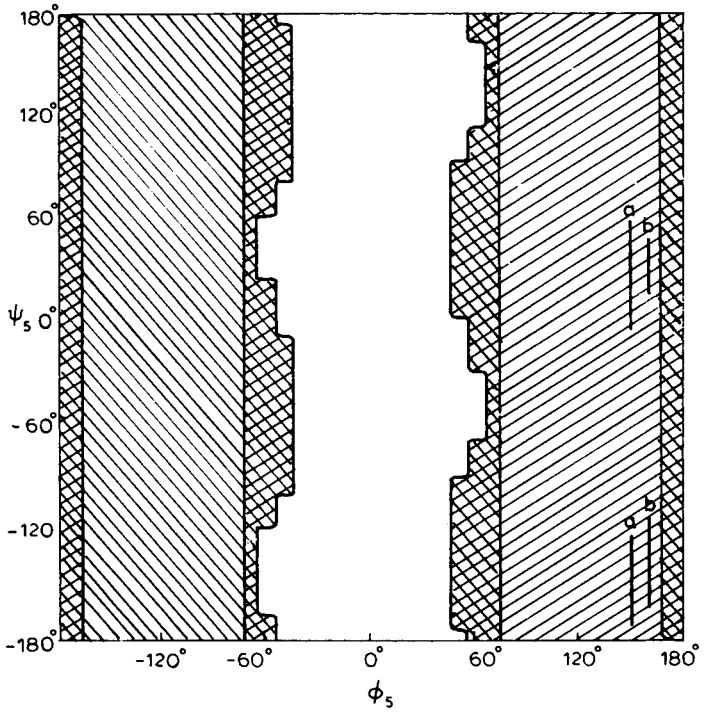

Fig. 4. Steric map for C-terminal alanine showing regions allowed for L-Ala (▨), D-Ala (▨), and both L- and D-Ala (▨). The vertical lines represent the allowed conformations of (a) penicillin G and (b) penicillin V.

Energy Calculations

As already mentioned, the terminal dipeptide segment of the pentapeptide should adopt a particular conformation for proper binding with the enzyme. To find the excess energy of this conformation from the global minimum, a search was first made to find out the global minimum for the dipeptide segments.

The total potential energy of the dipeptide segments is a function of four variables (ϕ_4, ψ_4) and (ϕ_5, ψ_5). It would be rather complicated and time-consuming to search the global minimum by considering all possible combinations of the two sets of dihedral angles. Hence, the Fletcher-Powell[15] modification of the Davidon[16] minimization method was followed to arrive at the global minimum. In this procedure a number of probable conformations were chosen with different sets of (ϕ_4, ψ_4) and (ϕ_5, ψ_5) angles and all four variables were simultaneously allowed to vary to reach the local minimum on the four-dimensional energy surface. Among the many local minima, the lowest one was considered as the global minimum.

The starting conformations of X-D-alanyl[4]-D-alanine[5] were selected as follows: the first five low-energy conformational angles of *N*-acetyl *N'*-methyl-L-alanyl amide[17] with sign changed (for D residue) were the starting sets for (ϕ_4, ψ_4). Four points (i.e., −60°, 60°, 80°, and 150°) were considered

for ϕ_5. In simple peptides in the solid state,[18] the ψ angle of the carboxyl group is closer to 0° or 180°. Hence, ψ_5 was initially chosen with these two values. A combination of the above values of (ϕ_4, ψ_4) and (ϕ_5, ψ_5) was the starting conformation. The starting conformations of X-D-alanyl^4-L-alanine^5 are also the same as that of X-D-alanyl^4-D-alanine^5, except for the change of sign in (ϕ_5, ψ_5).

For the other four analogs, X-D-butyl^4-D-alanine^5, X-D-alanyl^4-D-butyric acid^5, X-D-valyl^4-D-alanine^5, and X-D-alanyl^4-D-valine^5, (ϕ_4, ψ_4) were started at four points in the (ϕ, ψ) plane: two near the β-region, one near α_R, and the other near the α_L region. ϕ_5 was started at −60°, 60°, 100°, and 150°. ψ_5 was started at only one point, i.e., 30°, since in X-D-alanyl^4-D-alanine^5 most of the minimum-energy conformations have $\psi_5 \simeq 30°$. The calculations were done for all three staggered orientations of the side chain.

For the two sets of dihedral angles (ϕ_4, ψ_4) and (ϕ_5, ψ_5), ϕ_4 is flexible in different penicillins and can assume a wide range of values, although for optimal binding it should be around 180° (Ref. 8 and manuscript in preparation). Unlike ϕ_4, ψ_4 is fixed to a specific value (−128° in penicillin G) due to the rigid lactam ring. ϕ_5 can assume a range of values (110°–160°) due to different modes of thiazolidine ring-puckering, but the exact value required for optimal fit with the enzyme is not known. The fourth angle ψ_5 can assume a suitable orientation due to its higher flexibility. Hence, the conformational energy of X-D-alanyl^4-D-alanine^5 and its analogs X-D-alanyl^4-L-alanine^5, X-D-butyl^4-D-alanine^5, X-D-alanyl^4-D-butyric acid,^{5} X-D-valyl^4-D-alanine^5, and X-D-alanyl^4-D-valine^5 were calculated as a function of ϕ_4 (from −180° to +180° at 10° intervals), fixing ψ_4 at −128° (the value observed in penicillin G) and minimizing (ϕ_5, ψ_5) at each step. In the case of X-D-alanyl^4-L-alanine^5 and X-D-alanyl^4-D-valine^5 (for $\chi_5^1 = 60°$), the energy, with ϕ_5 fixed at 180° and 150°, respectively, was also calculated for the reasons given below. The results are shown in Figs. 5–9. The energy (V_{ex}) plotted in the y axis is the relative energy with respect to the global minimum of the molecule. The difference in energy ΔV_{ex} at $\phi_4 = 180°$ between the analog and X-D-alanyl^4-D-alanine^5 (substrate) and the observed percentage cross-linkage are given in Table I.

RESULTS AND DISCUSSION

Recent conformational energy calculations of penicillin indicate that its competitive inhibitory potency is due to the higher preference of the conformation $\phi_4 \simeq 180°$ in the inhibitor than in the substrate (manuscript in preparation). Also, it was found in the present calculations that in all the fragments, except X-D-alanyl^4-L-alanine^5 and X-D-alanyl^4-D-valine^5 (for $\chi_5^1 = 60°$), (ϕ_5, ψ_5) assume values around (150°, 30°) after minimization, which is very close to the values observed in penicillins G and V.[8] Hence, the relative activities of different analogs can be explained in terms of their relative preference for the conformation $\phi_4 \simeq 180°$ when ψ_4 is held fixed at −128° (the solid-state value in penicillin G).

TABLE I
Conformational Energy at $\phi_4 = 180°$ and Percentage Cross-Linking in Peptidoglycan Biosynthesis

Peptides	ΔV_{ex} (kcal/mol)[a]			Cross-linking[b] (%)
	χ_4^1 or χ_5^1 = 60°	= 180°	= −60°	
X-D-alanyl[4]-D-alanine[5]	0.0	—	—	100
X-L-alanyl[4]-D-alanine[5]	>10.0	—	—	0
X-D-alanly[4]-L-alanine[5]	>10 (4.12)[c]	—	—	14
X-D-butyl[4]-D-alanine[5]	>10.0	−0.68	6.42	5
X-D-alanyl[4]-D-butyric acid[5]	0.12	0.12	1.12	76
X-D-valyl[4]-D-alanine[5]	>10.0	6.02	>10.0	4
X-D-alanyl[4]-D-valine[5]	3.50	2.02	3.26	14
X-D-norvalyl[4]-D-alanine[5]	—	—	—	1
X-D-alanyl[4]-D-norvaline[5]	—	—	—	61

[a] ΔV_{ex} is the difference in energy between the analog and X-D-alanyl[4]-D-alanine[5] ($\Delta V_{ex} = V_{ex}^{analog} - V_{ex}^{substrate}$).

[b] Percentage cross-linking calculated from the data reported by Carpenter et al. (Ref. 6) and Marquet et al. (Ref. 7).

[c] The value within the parenthesis denotes the energy value when $\phi_5 = 180°$.

Conformational Specificity of the Fourth Residue

Among the various modifications made at the 4 position of the pentapeptide, a change of configuration has a dramatic effect and completely eliminates the cross-linking reaction (Table I). The preferred conformation of a L-alanyl residue is completely different from that of a D residue and the segment X-L-alanyl[4]-D-alanine[5] cannot assume conformations around $\phi_4 \simeq 180°$, $\psi_4 = -128°$.[8] Hence, when the configuration of the fourth residue is changed from D to L, the enzyme transpeptidase cannot bind with the terminal dipeptide segment to carry out the cross-linking reaction. This accounts for the total elimination of cross-linking for this analog.

From the steric map (Fig. 2) it is clear that when the D-butyl residue is substituted at the 4 position, only for one of the χ_4^1 angles ($\chi_4^1 = 180°$) is the biologically significant range ($\phi_4 \simeq 180°$, $\psi_4 \simeq -128°$) allowed. For the other two side-chain orientations ($\chi_4^1 = \pm 60°$), it is completely disallowed. It is interesting to see from Fig. 3 that with D-valine at the 4 position the required (ϕ_4, ψ_4) is sterically disallowed for all three orientations of the side chain. Hence, X-D-valyl[4]-D-alanine[5] cannot assume a favorable conformation to bind with transpeptidase, whereas X-D-butyl[4]-D-alanine[5] can assume such a conformation for one of the side-chain orientations. These results qualitatively explain the marked reduction in the cross-linking reaction (Table I) when D-valine or D-butyric acid is substituted at the 4 position of the pentapeptide. The reasoning given for D-butyric acid holds good for D-norvaline also, since the side chain of norvaline differs from that of butyric acid only at the δ-position. For a quantitative understanding of the effect of the D-butyl, D-valyl, or L-alanyl residue on the cross-linking reaction, conformational energies are discussed.

If the minimum-energy conformation of the substrate is around $\phi_4 \simeq 180°$ and $\psi_4 \simeq -128°$, optimal binding with the enzyme may occur without a loss of any binding energy for the complex formation. On the other hand, if the minimum-energy conformation of the substrate differs slightly from the one required for optimal binding, part of the binding energy may be utilized in bringing the substrate to the proper conformation from the minimum-energy conformation. In the case of X-D-alanyl[4]-D-alanine[5], the conformational energy is at a minimum when $\phi_4 \simeq 160°$ and $\psi_4 \simeq -160°$. From Fig. 5 it can be seen that the energy around $\phi_4 = 170°$–$180°$ (ψ_4 fixed at $-128°$) is about 1.5–3 kcal/mol. Hence, the enzyme may bind to the small population of the substrate which may exist in this conformation at equilibrium or it may undergo small conformational changes and bind with the low-energy conformers of the substrate which are closer to the required ones and subsequently twist them to $\phi_4 = 180°$ and $\psi_4 \simeq -128°$ for optimal fit. The energy required to twist the substrate in the latter case may be derived from the binding energy. The energy of X-D-valyl[4]-D-alanine[5] around $\phi_4 \simeq 180°$ is very high (Table I and Fig. 5) compared to that of X-D-alanyl[4]-D-alanine[5], and thus the population of the

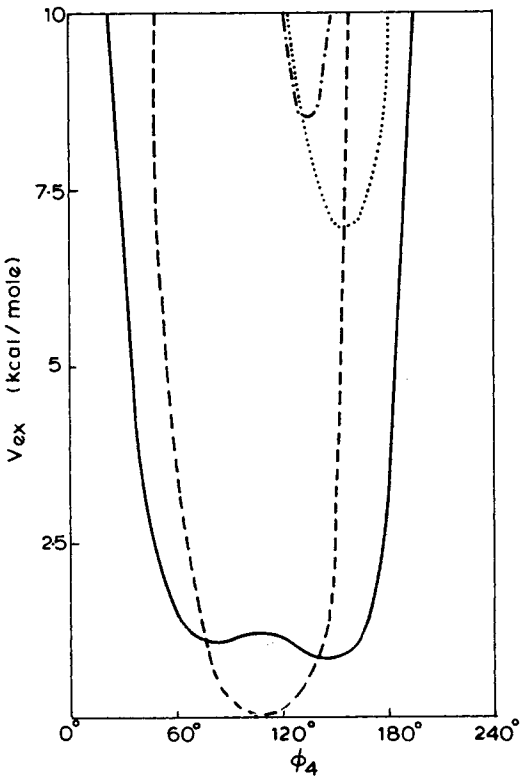

Fig. 5. Excess energy V_{ex} of X-D-valyl[4]-D-alanine[5] plotted against ϕ_4 for different side-chain orientations: - - - -, $\chi_4^1 = 60°$; · · · ·, $\chi_4^1 = 180°$; - · - · -, $\chi_4^1 = -60°$. The solid line represents the excess energy for X-D-alanyl[4]-D-alanine[5].

right conformers in the conformational equilibrium that can bind with the enzyme will be practically negligible. Even if the enzyme initially binds with the low-energy conformers which are closer to the required ones, it has to spend a relatively large amount of energy (Table I) to bring ϕ_4 to 180° and ψ_4 to −128°. This explains the drastic reduction in the cross-linking reactions when valine occupies the 4 position of the pentapeptide moiety.

In the case of X-D-butyl[4]-D-alanine[5], the energy around $\phi_4 \simeq 180°$ is very high (Table I and Fig. 6) for two of the butyl side-chain orientations ($\chi_4^1 = \pm 60°$) and is nearly the same as that of X-D-alanyl[4]-D-alanine[5] for the third orientation ($\chi_4^1 = 180°$). Hence, the *gauche* orientations of the side chain will drastically reduce the cross-linking and the effect of the *trans* orientation will be negligible. Thus, if equal probability is assumed for the three staggered orientations of the side chain, the cross-linking according to these calculations should be about 33% of that of X-D-alanyl[4]-D-alanine[5]. It can be seen from Table I, however, that the observed cross-linking is very low and the effect of the D-butyl or D-norvalyl residue at the 4 position is nearly the same as that of D-valine. One possible explanation for this discrepancy may be that the side group of the butyl residue in the *trans* conformation ($\chi_4^1 = 180°$) may not be accommodated in the active site of the enzyme.

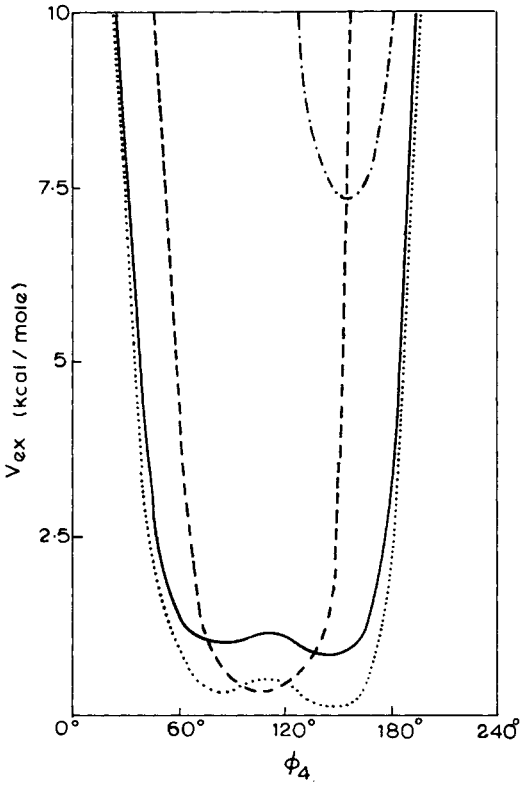

Fig. 6. Excess energy V_{ex} of X-D-butyl[4]-D-alanine[5] plotted against ϕ_4 for different side-chain orientations: - - - -, $\chi_4^1 = 60°$; · · · ·, $\chi_4^1 = 180°$; - · - · -, $\chi_4^1 = -60°$. The solid line represents the excess energy for X-D-alanyl[4]-D-alanine[5].

Conformational Specificity of the Fifth Residue

It can be seen from Fig. 7 that for the analog X-D-alanyl^4-D-butyric acid^5 the energy at $\phi_4 \simeq 180°$ for two orientations of the side chain ($\chi_5^1 = 60°$, 180°) is nearly the same as that of X-D-alanyl^4-D-alanine^5. For $\chi_5^1 = -60°$, it is only about 1 kcal/mol higher (Table I), suggesting a small reduction in the cross-linking for this orientation of the side chain. Hence, the cross-linkage for the analog X-D-alanyl^4-D-butyric acid^5 should be higher than 67% of the X-D-alanyl^4-D-alanine^5 cross-linkage. This is in good agreement with the experimental value of 76% (Table I). In the case of X-D-alanyl^4-D-valine^5, it can be seen from Fig. 8 that around $\phi_4 \simeq 180°$ the energy increases, compared to X-D-alanyl^4-D-alanine^5, by about 2–3 kcal/mol for $\chi_5^1 = 180°$ and $-60°$. For $\chi_5^1 = 60°$, it is nearly the same as that of X-D-alanyl^4-D-alanine^5. Hence, the cross-linking for the analog X-D-alanyl^4-D-valine^5 should be about 33%. A comparison with experimental results (Table I) reveals that the theoretical value is slightly higher. A critical examination of the results reveals that in X-D-alanyl^4-D-alanine^5 and some of its analogs (X-D-butyl^4-D-alanine^5, X-D-alanyl^4-D-butyric acid^5, and X-D-valyl^4-D-alanine^5), where theoretical predictions agree with the experimental results the favored conformation of the fifth residue is

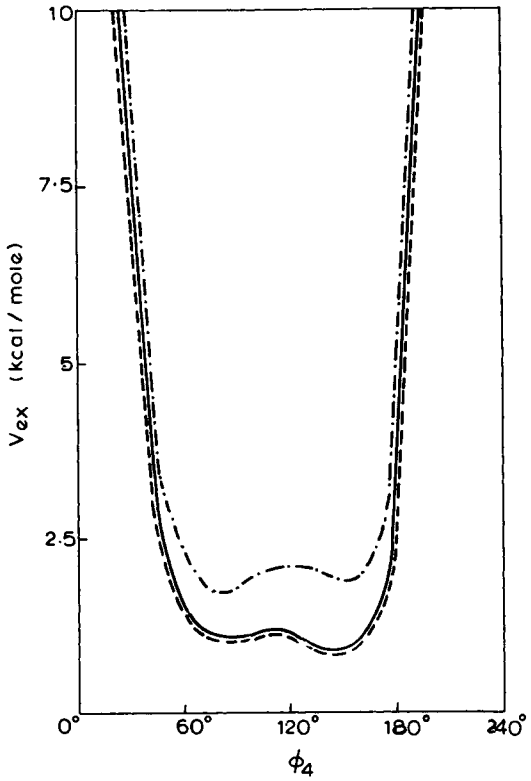

Fig. 7. Excess energy V_{ex} of X-D-alanyl^4-D-butyric acid^5 plotted against ϕ_4 for different side-chain orientations: - - - -, $\chi_5^1 = 60°$ and 180°; - · - · -, $\chi_5^1 = -60°$. The solid line represents the excess energy for X-D-alanyl^4-D-alanine^5.

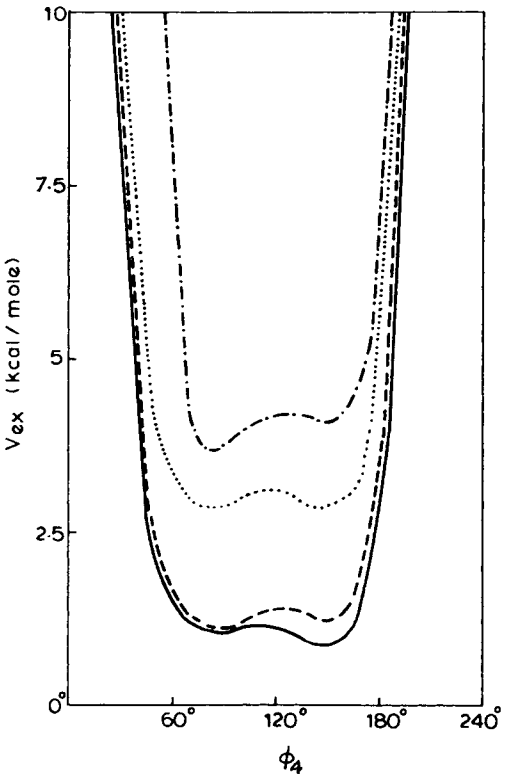

Fig. 8. Excess energy V_{ex} of X-D-alanyl^4-D-valine^5 plotted against ϕ_4 for different side-chain orientations: - - - -, $\chi_5^1 = 60°$; • • • •, $\chi_5^1 = 180°$; - • - • -, $\chi_5^1 = -60°$. The solid line represents the excess energy for X-D-alanyl^4-D-alanine^5.

around $\phi_5 \simeq 150°$ and $\psi_5 \simeq 30°$. In penicillins G and V, $\phi_5 \simeq 150°$ and $\psi_5 \simeq 30°$, also. In the analog X-D-alanyl^4-D-valine^5, the favored value of ϕ_5 is about 150° for two of the orientations of the side chain ($\chi_5^1 = 180°$ and $-60°$) and about 80° when $\chi_5^1 = 60°$. It should be recalled that ΔV_{ex} at $\phi_4 \simeq 180°$ is negligible for $\chi_5^1 = 60°$. When the energy of X-D-alanyl^4-D-valine^5 was calculated for $\chi_5^1 = 60°$, fixing ϕ_5 at 150°, ΔV_{ex} increases to 3.5 kcal/mol (Table I). Hence, for all three orientations of the side chain, the energy ΔV_{ex} is high (2–3 kcal/mol), which accounts for the reduction in cross-linkage to 14%.

When no constraint was imposed on the dihedral angles ϕ_5 and ψ_5 for the analog X-D-alanyl^4-L-alanine^5, the energy curve was found to be identical to that of X-D-alanyl^4-D-alanine^5. But the favored conformation of the fifth residue is different; in X-D-alanyl^4-D-alanine^5, $\phi_5 \simeq +150°$; whereas in X-D-alanyl^4-L-alanine^5, $\phi_5 \simeq -150°$. When the energy of X-D-alanyl^4-L-alanine^5 was also calculated with ϕ_5 fixed at +150°, the energy increases steeply. At $\phi_4 \simeq 180°$, ΔV_{ex} is more than 10 kcal/mol. However, when the calculations were done fixing ϕ_5 in the commonly allowed range +170° to −170° (Fig. 4), ΔV_{ex} decreases. The energy curve corresponding to $\phi_5 \simeq 180°$ is given in Fig. 9. From Fig. 9 and Table I it can be seen that

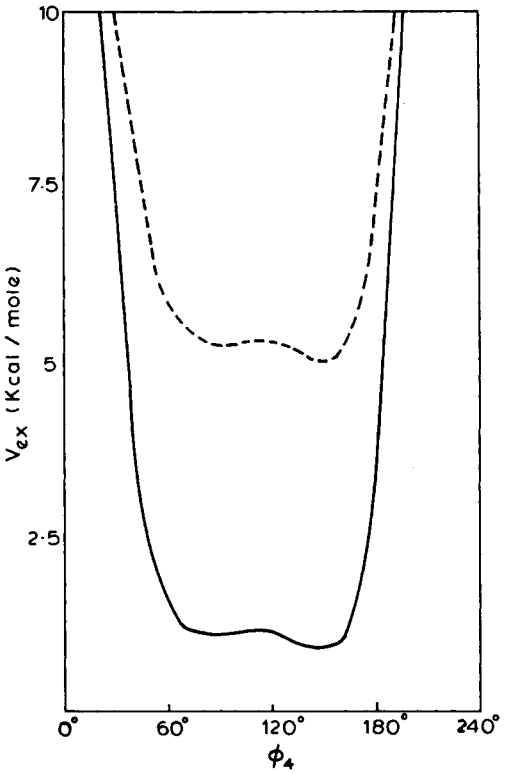

Fig. 9. Excess energy V_{ex} of X-D-alanyl[4]-L-alanine[5] plotted against ϕ_4: - - - -, $\phi_5 = 180°$. The solid line represents the excess energy for X-D-alanyl[4]-D-alanine[5].

ΔV_{ex} at $\phi_4 \simeq 180°$ is about 4 kcal/mol. Thus, the probability of X-D-alanyl[4]-L-alanine[5] existing in a conformation closer to the biologically active conformation of X-D-alanyl[4]-D-alanine[5] is small and the cross-linkage should therefore be very low. The above prediction is in agreement with the experimental results (Table I). The specific value of ψ_5 required for optimal binding in the above analogs is not stressed, since it can assume a suitable value due to its higher degree of conformational freedom.

CONCLUSION

The enzyme transpeptidase thus requires a specific conformation ($\phi_4 \simeq 180°$, $\psi_4 \simeq -128°$, and $\phi_5 \simeq 150°$) of the fourth and fifth residues of the pentapeptide moiety for optimal binding. Configurational change or substitution at these positions by residues containing a C^γ atom or beyond in the side chain affects the above conformation. Perhaps this may be the reason for the occurrence of X-D-alanyl[4]-D-alanine[5] in peptidoglycans of different species.

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