A molecular mechanics study on conformations of bacterial polyester poly-(3-hydroxybutyrate-*co*-3-hydroxyvalerate)

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The conformational properties of bacterially synthesized copolyesters of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV), P(3HB-*co*-3HV), have been studied using the MM2 molecular mechanics method. The MM2 calculation is applied to some model compounds having carefully selected initial geometries and finite chain length. The present calculation reveals that the isolated copolymer chain of P(3HB-*co*-3HV) has some types of stable 2_1 helices over the whole comonomer composition range, and that the distortion induced on the helical conformation is much greater when 3HV units are incorporated into the 3HB helix than when 3HB units are incorporated into the 3HV helix. On the basis of these results, the crystalline morphology is discussed.

(Keywords: poly(3-hydroxybutyrate); poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); copolyester; molecular mechanics; conformation; crystal)

INTRODUCTION

A variety of micro-organisms are known to accumulate poly-3-hydroxybutyrate P(3HB), an optically active polyester, as an intracellular storage polymer[1,2]. This polyester is stored under conditions including a sufficient amount of carbon sources but lacking one of the known growth factors such as nitrogen, phosphorus and oxygen. Recently various bacterial polyesters of this family, namely poly-3-hydroxyalkanoate, have been biosynthesized and the copolyesters of 3-hydroxybutyrate and 3-hydroxyvalerate, P(3HB-*co*-3HV), have been widely studied[3–5]. The physical properties of these copolymers vary drastically depending on their 3HV composition.

Bluhm *et al.*[6] reported that the curve of melting point *versus* 3HV composition for the range of 3HV compositions from 0 to 47% does not follow Flory's equation for the melting point of copolymers[7]. The theory assumes that the minor components are excluded from the crystal. Consequently, they proposed that both of the comonomers cocrystallize in the P(3HB) crystalline lattice forming isomorphous crystals.

On the other hand, Mitomo *et al.* reported that the melting point of copolyesters with 3HV compositions of 0–30% follow Flory's equation, and that the 3HV units tend to be excluded from the P(3HB) crystal although some are included and act as defects[8].

Recently, we have shown by high resolution solid state n.m.r.[9] that large amounts (up to 14%) of HV units are included in the P(3HB) crystalline lattice. Similarly, P(3HB) units are included in the P(3HV) lattice even when the HB content is relatively high. According to X-ray analysis[10,11], the P(3HB) and P(3HV) homopolymers exhibit 2_1 helix structures in the crystalline state. In HB- and HV-rich copolymers, the X-ray diffraction pattern does not alter with comonomer composition, although *d*-spacings undergo slight modifications. These results strongly support the possibility of co-crystallization.

In general, the ease of co-crystallization depends on the degree of structural similarity between the major and minor components. It is at least necessary that the incorporation of the minor component causes no appreciable distortion in the backbone structure of the parent homopolymer chain. The side chain of an HV unit is more bulky than that of an HB unit by one methylene group. It is of great interest to investigate how the backbone structures of the homopolymers P(3HB) and P(3HV) are modified by the incorporation of HV and HB units, respectively. However, the experimental data alone provide no direct information on the local structure of the backbone containing the minor components.

In this paper, we report on the conformational aspects of P(HB-HV) copolymers using the MM2 molecular mechanics method[12]. It is shown that the copolymer chains take 2_1 helix structures similar to those of the parent homopolymers even when the minor component content is high.

COMPUTATIONAL DETAILS

Calculations were carried out for model compounds having finite chain lengths, which were, as will be described later, determined by examining the dependence of their geometrical parameters and steric energy on the number of monomer units included. The MM2 energy minimization was applied to each model compound in

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order to find its stable conformation. The reliability of the MM2 method was confirmed by comparing the optimized geometries of the PHB and PHV homopolymer models with the corresponding X-ray geometries. Three different X-ray structures for P(3HB) have been reported[10,13,14]. They are denoted as X-ray structures A, B and C, respectively. The MM2 minimization was carried out for each X-ray structure.

The main purpose of this study is to investigate whether or not the copolymer chain with a high comonomer content can have stable conformations similar to those of the corresponding parent homopolymers. Thus, in copolymer models, their initial conformations were also determined by reference to the X-ray geometries of the P(HB) and P(HV) homopolymers. The assumed conformations are as follows: (1) all the rotational angles around the backbone bonds are taken to be equal to the X-ray data for either of the homopolymers; (2) the backbone conformations of major and minor components are taken to be equal to the X-ray data for their own homopolymers. In the first case, all repeating units have the same backbone conformations irrespective of their comonomer type, whereas in the second case the backbone conformation is variable for each repeating unit according to its comonomer type. The former is divided into two cases according to their geometrical origins, namely, P(3HB) or P(3HV). Hereafter, the P(3HB)- and P(3HV)-type backbones are called structures I and II, respectively. The initial geometry of the second case is called structure III. The monomer units and rotational angles are shown in *Figure 1*. The values of the rotational angles are taken from structures A, B or C for structure I (*Table 1*) and $\psi = -60°$, $\phi = -21°$, $\omega = 179°$, $\theta = 136°$ for structure II. For structure III, the former set of rotational angles are employed for 3HB units and the latter for 3HV units.

In the P(3HV) model, the side chain conformation can be specified by the angle ε, defined as the rotational angle around the carbon–carbon single bond connecting the backbone methine and side chain methylene groups as shown in *Figure 1*. The value of ε was so determined as to be zero when the methyl carbon is in the *cis* form with respect to the ester oxygen of the backbone.

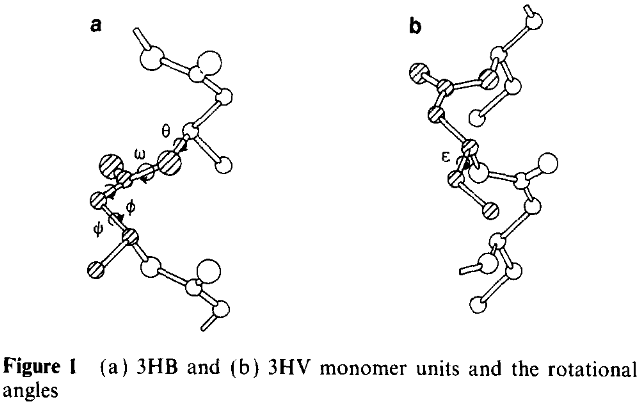

Figure 1 (a) 3HB and (b) 3HV monomer units and the rotational angles

The initial values for bond angles and bond lengths were taken from the corresponding natural (non-strained) geometries used in the MM2 method.

RESULTS

Construction of model compounds

We first attempted to obtain the optimized structure of the P(3HB) model starting from X-ray structure A[10]. *Figure 2* shows the chain length dependence of the optimized rotational angles. With an increase in chain length, each rotational angle rapidly converges. The deviation from the X-ray value is ±5° in each case. The value of the helix pitch also converges around the experimental value as chain length increases (*Figure 3*). Thus, if chain length is taken to be > 10 units, the X-ray structure is satisfactorily reproduced by the MM2 calculation. In the model compound having 15 monomer units, B15, a regular structure such as a 2_1 helix is formed in the region extending from the 3rd to the 12th unit

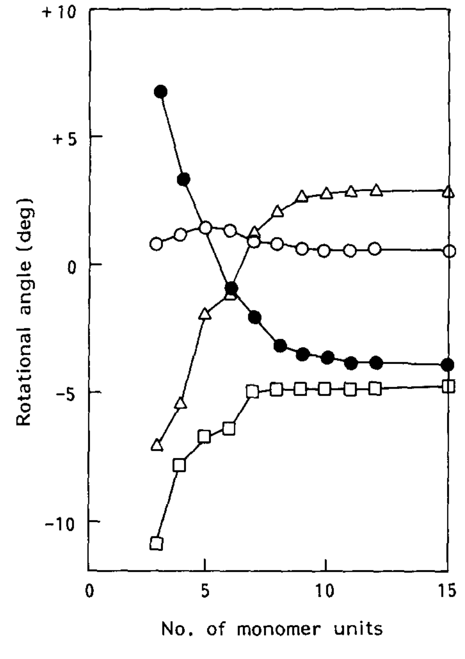

Figure 2 Dependence of average rotational angles on chain length of the model compounds when optimized from structure A. The values for the rotational angles are the deviation from the X-ray structure A. Rotational angles: (○) ψ; (△) ϕ; (□) ω; (●) θ

Table 1 Rotational angles of the P(3HB) backbone determined using three types of X-ray diffraction data and those calculated from the corresponding model compounds

	X-ray data[a] (deg)				Calculated[b] (deg)				
	ψ	ϕ	ω	θ	ψ	ϕ	ω	θ	Energy (kJ mol^{-1})
A	−52	−42	−175	152	−51.3	−39.2	−179.7	148.4	92.8
B	−57	−31	180	142	−58.8	−17.8	−175.2	129.9	95.4
C	−59	−35	−173	149	−53.9	−30.0	−177.6	141.3	94.3

[a] Data for A, B and C were taken from references 10, 13 and 14, respectively
[b] Rotational angles of the central (no. 8) monomer unit

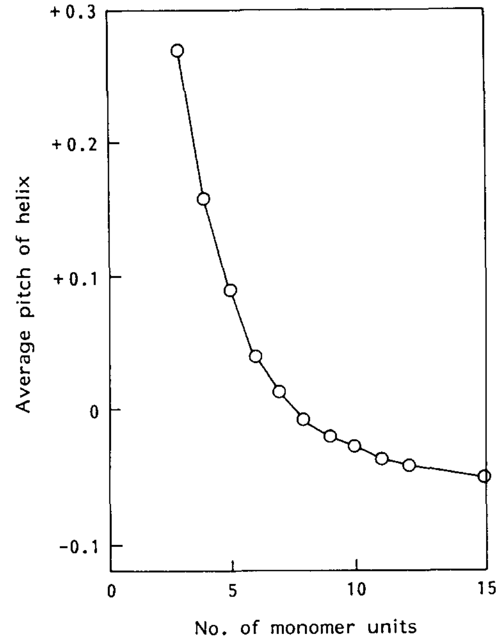

Figure 3 Average pitch of helix *versus* the number of monomer units when optimized from structure A. The values for pitch are the deviation from the X-ray data

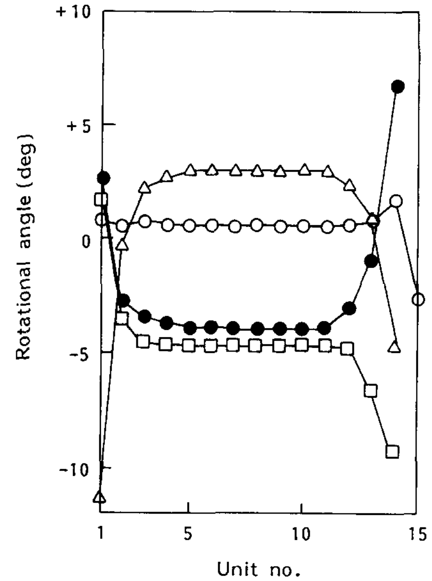

Figure 4 Each rotational angle along the backbone in B15 after optimization from the initial X-ray structure A *versus* the monomer unit number. A zero value for the rotational angle corresponds to the X-ray structure A. Symbols as in *Figure 2*

(*Figure 4*). This region is regarded as a better analogue of the P(3HB) homopolymer.

Similar calculations were also carried out for X-ray structures B[13] and C[14]. The final results for the B15 models are summarized in *Table 1*. Structures A and C are well reproduced by calculation, whereas in the case of structure B the calculated values considerably deviate from the experimental values, especially for the angles θ and ϕ, and also from the other calculated values obtained from structures A and C. The extent of the regular structure range also showed the superiority of structure A over structures B or C. These results may be understood by considering that structure B involves energetically unfavourable structures (discussed in reference 13). Thus, structure B might be inappropriate as an initial structure for the P(3HB) polymer. Owing to the geometrical similarity of structures A and C, either structure can be employed. Here, we use structure A, giving the optimized structure with lower steric energy.

In the same way, the model compound of the P(3HV) homopolymer was constructed. *Figure 5* shows the chain-position dependence of each rotational angle in the model compound having 15 monomer units (V15). The regular structure along the backbone is again found in the region from the 3rd to the 12th monomer unit and in this region the deviations of the calculated rotational angles are within ±3° of the corresponding experimental values. As for the rotational angles of side chains, ε, only a deviation of ±0.5° around 66° was shown. These results indicate the sufficient accuracy of the MM2 calculation for the P(3HV) homopolymer. V15 is thus appropriate as a model of P(3HV).

Conformational analysis of copolymer models

3HB-rich copolymer models (*structure I*). The model compounds of 3HB- and 3HV-rich copolymers were constructed by modifying the homopolymer models B15 and V15, respectively. Their backbone conformations were determined as described above. First, the central (8th) unit of B15 was replaced by an HV unit and the initial backbone conformation was taken as structure I. The rotational angle ε was taken to be 64°, which is equal to the value of the X-ray structure of P(3HV). This copolymer model is denoted as B14V1. This method of introducing HV units into the P(3HB) model compound is shown to be appropriate by the following calculation. The B14V1 model compounds with four initial values for ε (0, 60, 120, 180°) are optimized to almost the same conformational structures (*Table 2*). There seems to be no appreciable difference among them within calculation error. The optimized rotational angles are shown in *Figure 6*. Next, we direct our attention to conformational changes in the region from the 3rd to the 12th unit, corresponding to the regular structure region of the parent homopolymer model B15. At the 7th unit, the rotational angles ϕ and θ change by +11.4° and −11.1°

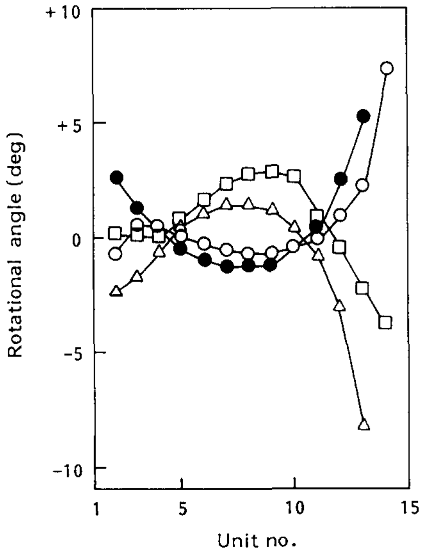

Figure 5 Each rotational angle along the backbone in V15 after optimization *versus* the monomer unit number. A zero value for the rotational angle corresponds to the X-ray data for P(3HV). Symbols as in *Figure 2*

Table 2 Four initial values of ε and the resulting conformational structures

Rotational angle ε (deg)		Backbone rotational angles^a (deg)			
Initial	Optimized	ψ	ϕ	ω	θ
0	65.2	−51.8	−38.4	−179.8	147.8
60	65.1	−51.8	−38.1	−179.2	146.7
120	65.2	−51.9	−38.2	−179.7	147.7
180	66.2	−51.8	−38.5	−179.7	148.3

^{a}The values are the average of the 3rd to 12th monomer units

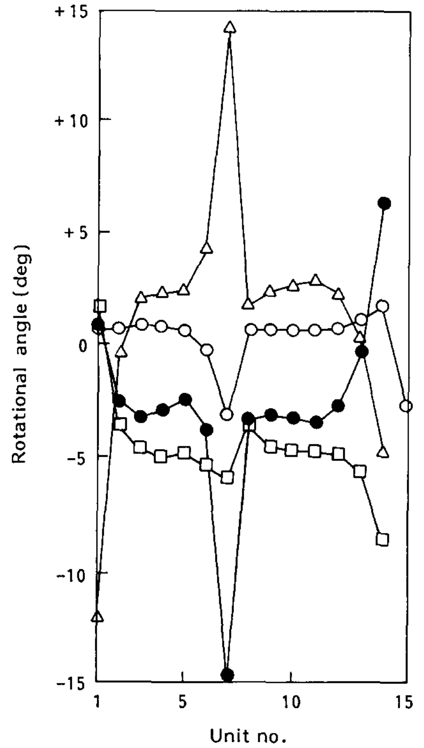

Figure 6 Each rotational angle along the backbone in B14V1 after optimization *versus* the monomer unit number. A zero value for the rotational angle corresponds to the X-ray data for P(3HB). The most remarkable changes occur around the carboxyl atoms nearest the ethyl side group of the HV monomer unit. Symbols as in *Figure 2*

from those of B15, respectively. Such large changes are not found in the other units. The angles ψ and ω undergo slight modifications. These characteristic changes in the backbone conformation were independent of the position of HV substitution. Consequently, the substitution of one comonomer, going from HB to HV, influences only the angles ϕ and θ of the nearest neighbouring HB unit located on the side of the smaller unit number.

This conformational change is attributed to the steric interaction between the ethyl side group of HV and the nearest carboxyl group. The steric energy of B14V1 before optimization was separated into the atom–atom pair interactions (*Table 3*). The overall increase in steric energy by changing one monomer unit from 3HB to 3HV is given in *Table 3*. The steric interactions can be relaxed by allowing rotation around the angles ϕ and θ of the 7th unit only, being supported by the CPK molecular model. The conformational distortion induced by incorporating the HV unit is localized in the vicinity of the substituted unit and not propagated to longer distant units. As a result, the 2_1 helix structure of the parent homopolymer is almost unchanged in the B14V1-type copolymer models.

Next, two types of disubstituted copolymer models, B13V2, were examined in the same way. The HV units were introduced at either the 7th and 8th units or the 6th and 8th units. The resulting structures are shown in *Figures 7a* and *b*. In each case, the conformational changes are found only in the two HB units nearest to the HV units. The results indicate that the properties of the resulting structures should not depend on the position of substitution.

Calculations were performed for trisubstituted copolymer models with various patterns of HV distribution. As can be predicted from the results for the mono- and disubstituted models, the 2_1 helix structure of the parent homopolymer is unchanged, and no dependence of the structure on the position of substitution was shown.

Therefore, it can be concluded that the 2_1 helical structure is stable even with considerably higher HV content as long as the initial geometry is taken as structure I.

3HV-rich copolymer models (structure II). The 3HV-rich copolymer models were examined in the same way as for structure I. The central (9th) unit of V15 was replaced by an HB unit and its initial backbone conformation was taken as structure II. This copolymer model is denoted as V14B1. The optimized rotational angles of V14B1 are plotted against the unit number in *Figure 8*. It should be noted that no apparent change in the backbone conformation occurs around the 3HB monomer unit in contrast to the results for structure I. This indicates that the substitution of an HB unit for an HV unit, namely the substitution of the methyl side chain for an ethyl side chain, causes no significant steric hindrance on the backbone of the model compound.

Calculations for model compounds of di- and trisubstituted copolymers with various distributions of 3HB units revealed that no significant conformational change occurs by incorporating 3HB units into the 3HV helix.

It is concluded that HB units can be incorporated into the HV-rich copolymer models without any appreciable distortion of the backbone conformation when the MM2 optimization starts from structure II.

Structure III. The model compounds for copolymers with various 3HV compositions and distributions were optimized from structure III. All calculations produced folded chains which no longer maintained the uniformly extended 2_1 helical structure, even though steric energies calculated for these structures were comparable to those for structures I and II. For example, as for B14V1 in

Table 3 Van der Waals interactions between the CH_3 atoms of the ethyl side group of the 3HV unit and the C(1), O(1), O(2) atoms of the nearest carboxyl group

Interacting atoms	Interactions (kJ mol^{-1})
C(1)-CH_3	13.5
O(1)-CH_3	2.1
O(2)-CH_3^{a}	19.2
	34.8
Overall increase	35.9

^{a}O(2) corresponds to the carbonyl oxygen atom

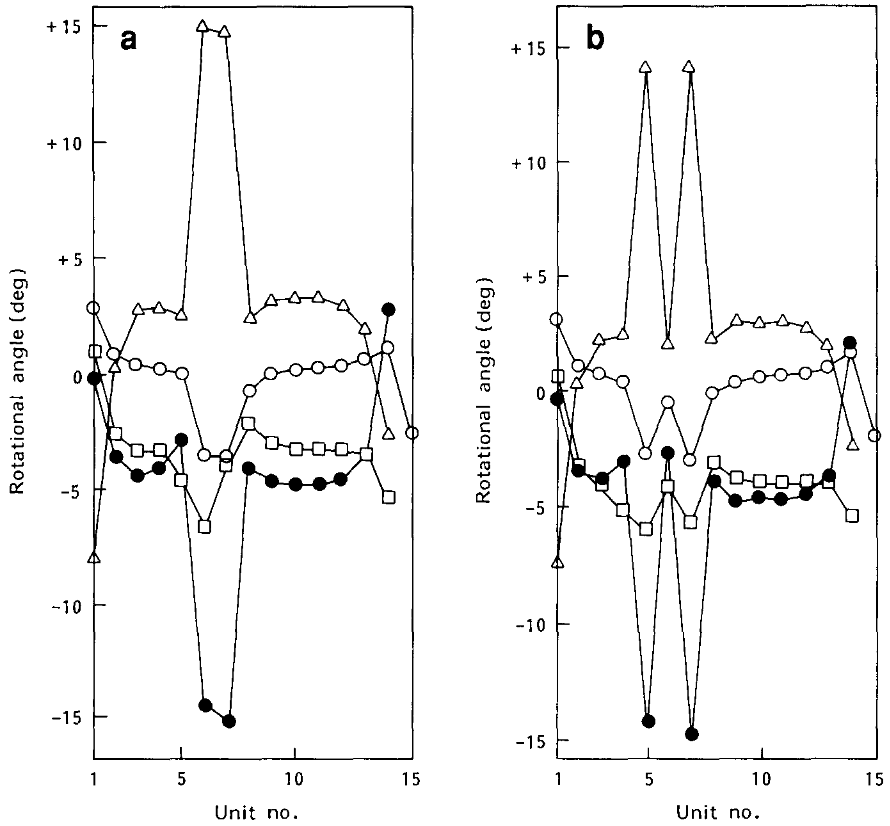

Figure 7 Each rotational angle along the backbone in disubstituted B13V2 after optimization *versus* the monomer unit number. A zero value for the rotational angle corresponds to the X-ray data for P(3HB). (a) HV units introduced at the 7th and 8th units. (b) HV units introduced at the 6th and 8th units. Symbols as in *Figure 2*

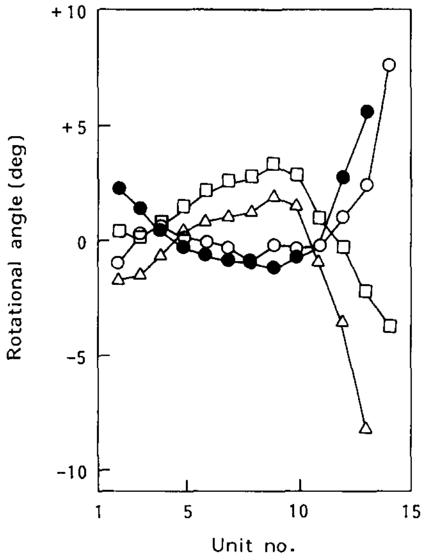

Figure 8 Each rotational angle of the backbone in V14B1 after optimization *versus* the monomer unit number. A zero value for the rotational angle corresponds to the X-ray data for P(3HV). Symbols as in *Figure 2*

which the 8th monomer unit is replaced by an HV unit, the resulting rotational angles ψ, ϕ and θ of the 7th unit deviate $-8°$, $+22°$ and $-8°$ from the average of the other units, respectively, while the other rotational angles remain unchanged. This result indicates that the steric hindrance is localized around the substituted HV unit. For B1V14, in which the 8th HV unit is replaced by an HB unit, the optimized rotational angles ϕ of the 7th and 8th units deviate $+17°$ and $-14°$ from those of the other units, respectively, and no significant variation is seen in the rotational angles at the other positions.

These results indicate that the different types of backbone structures are incompatible in one copolymer chain. Consequently, there is no stable 2_1 helical structure around structure III.

DISCUSSION

The conformational properties of the model compounds of P(3HB-*co*-3HV) have been studied starting from three types of initial structure. The final structures from structures I and II were similar to the 2_1 helices of the respective parent homopolymers, while that from structure III was not. The difference of the total steric energies between structures I and II is plotted against comonomer composition in *Figure 9*. In the range of 3HB-rich compositions (HV fraction 0–60%), type I chains are more stable. On the other hand, in the range of 3HV-rich compositions (HV fraction 60–100%), structures I and II are energetically comparable. It should be noted that in both chains the conformational energies are almost invariant with comonomer composition in the range of higher HV content (60–100%). The minor component, HB unit, behaves like a major component in this composition range. This is consistent with the fact that the backbone conformation is not explicitly perturbed by incorporating HB units into the structure II chain. Considering these results, it is expected that incorporation of minor components may cause no apparent influence on the crystalline morphology. In fact, according

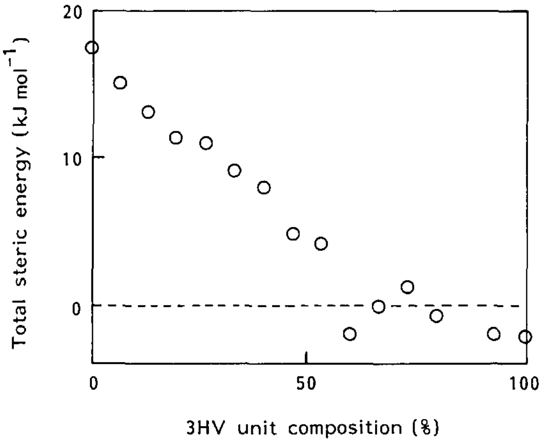

Figure 9 Difference in the total steric energies for those calculated for structures I and II. The values are calculated by subtracting the energies for structure II from those for structure I

to X-ray analysis, the lattice parameters are almost independent of comonomer composition in the range of higher HV content. On the other hand, the incorporation of HV units into the HB-type chain causes substantial perturbation to the parent homopolymer chain in both geometry and energy, even though the 2_1 helix structure is kept unchanged. This may account for gradual changes in the lattice parameters with comonomer composition.

The above argument ignores the effect of interchain interactions, which are difficult to include in calculations with sufficient accuracy. An alternative method for estimating such effects is to analyse the melting behaviour of the P(3HB-*co*-3HV) crystals on thermodynamic grounds. Recently, we have successfully evaluated the nearest neighbour interactions between comonomer units in the crystalline state[15]. That study enabled us to provide a more quantitative explanation for the crystalline morphology of P(3HB-*co*-3HV) copolymers.

The folded-chain generated from structure III has a conformational energy comparable to those of the 2_1 helices from structures I and II. In solution, the fast interconversion between these structures should be observed. However, in the crystalline state, such folded sites would be easily unfolded giving a straight helix, because the interchain interactions preferentially stabilize the helices arranged in parallel. On the basis of the present results, there are no specific sites or specific comonomer distribution patterns preferentially inducing folded structures in the copolymer chain. This supports the validity of our previous theoretical treatment of P(3HB-*co*-3HV) crystals, which treats a copolymer crystal as a metal–alloy system[15].

In conclusion, the present calculation revealed that the isolated copolymer chain of P(3HB-*co*-3HV) has some stable 2_1 helical structures over the whole composition range and that the conformational disturbance is much greater when 3HV units are incorporated into the 3HB helix than when 3HB units are incorporated into the 3HV helix.

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