J|A|C|S
ARTICLES
Published on Web 09/17/2004

Elastic Effects behind Cooperative Bonding in β-Sheets

Jan Rossmeisl, Jens K. Nørskov,* and Karsten W. Jacobsen

Contribution from the Center for Atomic-scale Materials Physics, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark

Received February 19, 2004; E-mail: norskov@fysik.dtu.dk

Abstract: We present extensive density functional theory calculations of the bonding between strands in β-sheets. We identify a significant cooperative effect whereby the interaction increases in strength with the number of strands. We show that the effect is related to a coupling between interstrand bonding and intrastrand elastic properties. It is found that a direct consequence of this coupling is that the pitch of β-sheets should contract with increasing number of strands, and we show that the effect can be observed directly in experimental data from the Protein Data Bank.

Introduction

β-sheets consist of polypeptide strands held together by hydrogen bonds. Typically, they consist of 2−6 strands of polypeptides and there are 5−10 residues in each strand, but larger sheets are present in some native proteins, for example, immunoglobulin and triose phosphate isomerase.[1] Experiments on small designed proteins suggest that the formation of β-sheets is aided by a cooperative effect making the strength of the interaction between the polypeptide strands increase with the number of strands.[2−5] The size and origin of the cooperative nature of the bonding in β-sheets is not understood although cooperativity may be crucial to the formation of molecular motifs and to the formation of β-amyloid fibrils as observed in connection with mad-cow disease and Alzheimer's disease.[6]

A cooperative bonding effect is a signature of a many-body interaction between the strands; the strength of a bond depends on the number of other bonds formed. It has been suggested that the formation of hydrogen bonds induces a charge polarization within the backbone of the strand, which makes it better suited for making additional hydrogen bonds.[7] Cooperative polarization and quantum effects are well described for the interactions between molecules of water,[7] and cooperative resonance effects are important for clusters of small amide molecules interacting through a single peptide hydrogen bond.[8,9] Also in α-helix structures, a cooperative effect has been identified.[10,11] However, recent density functional studies on model β-sheets containing polypeptides of Gly find no significant effect.[12] Other suggestions on the origin of the cooperative effect include hydrophobic clustering of side chains,[13] solvation, and structural effects.[14]

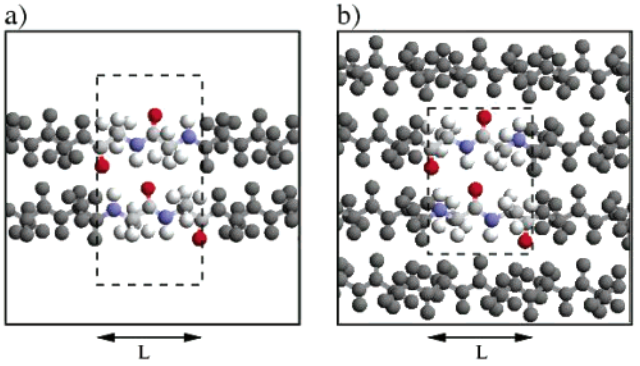
In the present paper, we show that there is a sizable cooperative effect in the bond energy between strands in β-sheets. On the basis of an extensive set of density functional theory calculations, we show that the cooperative effect originates from an interplay between the elastic properties of the single strands and the hydrogen bonds between strands. The calculations have the additional prediction that the broadest sheets with the strongest bonds must have the shortest pitch. We show that this effect can be observed in statistical data based on protein structures from the Protein Data Bank. The cooperativity we find is intrinsic in the sense that it is related to the peptides themselves. Effect from water or other part of the protein is not investigated in this study.

Method

All calculations are performed at the level of ab initio density functional theory GGA-(PW91). This functional performs well on hydrogen-bonded systems.[15,16] We use the plane wave, pseudopotential code *Dacapo*.[17] with periodic boundary conditions. One advantage of the plane wave basis set is that convergence is controlled by a single parameter, the cutoff energy. By setting the cutoff energy sufficiently high, convergence can be ensured. In this work, the cutoff energy is 25 Ryd, at which binding energies are converged within 1 kJ/mol. The calculations employ ultrasoft pseudopotentials,[18] a Fermi smearing of 0.001 eV, and Pulay mixing is used to obtain the self-consistent electron density.[19] All atomic structures are relaxed using a conjugated gradient

(1) Schulz, G. E.; Schirmer, R. H. *Principles of Protein Structure*; Springer-Verlag: New York, 1978.
(2) Kortemme, T.; Ramirez-Alvarado, M.; Serrano, L. *Science* **1998**, *281*, 253−256.
(3) Sharmann, G. J.; Searle, M. S. *J. Am. Chem. Soc.* **1998**, *120*, 5291−5300.
(4) Griffiths-Jones, S. R.; Searle, M. S. *J. Am. Chem. Soc.* **2000**, *122*, 8350−8356.
(5) Schenck, H. L.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4869−4870.
(6) Prusiner, S. B. *Science* **1997**, *278*, 245−251.
(7) Jeffrey, G. A. *An Introduction to Hydrogen Bonding*; Oxford University Press: New York, 1997.
(8) Kobko, N.; Paraskevas, L.; del Rio, E.; Danneberg, J. J. *J. Am. Chem. Soc.* **2001**, *123*, 4348−4349.
(9) Guo, H.; Gresh, N.; Roques, B. P.; Salahud, D. R. *J. Phys. Chem. B* **2000**, *104*, 9746−9754.
(10) Ireta, J.; Neugebauer, J.; Scheffler, M.; Rojo, A.; Galvàn, M. *J. Phys. Chem. B* **2003**, *107*, 1432−1437.
(11) Wu, Y. D. A.; Zhao, Y. L. *J. Am. Chem. Soc.* **2001**, *123*, 5313−5319.
(12) Zhao, Y. L.; Wu, Y. D. A. *J. Am. Chem. Soc.* **2002**, *124*, 1570−1571.
(13) Fersht, A. *Structure and Mechanism in Protein Science*; W. H. Freeman and Company: New York, 1998.
(14) Guo, C.; Cheung, M. S.; Levine, H. *J. Chem. Phys.* **2002**, *116*, 4353−4365.
(15) Tsuzuki, S.; Lüthi, H. P. *J. Chem. Phys.* **2000**, *114*, 3949−3957.
(16) Hamann, D. R. *Phys. Rev. B.* **1997**, *55*, 10157−10160.
(17) The DFT calculations were performed with the Dacapo pseudopotential code, which is available at http://www.fysik.dtu.dk/campos.
(18) Vanderbilt, D. *Phys. Rev. B.* **1990**, *41*, 7892−7895.
(19) Kresse, G.; Furthmüller, J. *J. Comput. Mater. Sci.* **1996**, *6*, 15−50.

10.1021/ja0490626 CCC: $27.50



Gly	a) E_2^{Bond}	b) E_∞^{Bond}	ΔE^{Bond}
⇉	−26 kJ/mol	−35 kJ/mol	−9 kJ/mol
⇄	−32 kJ/mol	−37 kJ/mol	−5 kJ/mol
Ala			
⇉	−27 kJ/mol	−38 kJ/mol	−11 kJ/mol
⇄	−31 kJ/mol	−39 kJ/mol	−8 kJ/mol
Val			
⇉	−41 kJ/mol	−48 kJ/mol	−7 kJ/mol
⇄	−43 kJ/mol	−50 kJ/mol	−7 kJ/mol

Figure 1. Top: The structures for antiparallel Ala strands. The calculations are performed on one period of the β-sheet, i.e., two residues, indicated by the colored atoms in the dashed box. Periodic boundary conditions are used. The length of the period along the polypeptides (the pitch of the sheet) is denoted L. (a) The structure of an interacting pair of strands. (b) The structure of an infinite sheet. Bottom: Calculated value of the binding energy between two strands interacting through two peptide hydrogen bonds is $E^{bond}{}_2 = E_2(L_2) - 2E_1(L_1)$ and the binding energy per strand in the infinite sheet is $E^{bond}{}_\infty = 1/2(E_\infty(L_\infty) - 2E_1(L_1))$, where $E_N(L_N)$ is the total energy of the N-stranded sheet at its equilibrium pitch L_N. The convention is such that a more negative value of the energy corresponds to a stronger bond. The difference between $E^{bond}{}_2$ and $E^{bond}{}_\infty$ is denoted ΔE^{bond}, and it is a measure of the cooperativity.

algorithm until the square root of the sum of the absolute forces is less than 0.05 eV/Å.

Unlike earlier such calculations, we do not only consider terminated polypeptide molecules, but also straight, infinitely long periodic polypeptide strands. This means that the ends of the strands do not influence the results. This periodic model has previously been found to reproduce hydrogen bond geometries of real β-sheets[20] and even the statistical propensities.[21] The periodicity restricts us to consider only a few different amino acids at a time in each polypeptide, and the model cannot account for any twisting of the sheets on longer length scales. We show later that the energy related to twisting is negligible. The strands are free to shear[22] relative to each other. The simulation set up is shown in Figure 1. We have studied the bonding between single-type polypeptides consisting of Ala, Val, and Gly. For each type of amino acid, five different molecular structures are used: the isolated single strand, an interacting pair of strands arranged both parallel and antiparallel to each other, and infinitely wide sheets in parallel and antiparallel structures. The single strand and the pair of strands are simulated in a box, which is 15 Å in the directions perpendicular to the strand, which is large enough to ensure no interactions between pairs of strands. The length of the box along the strands is denoted L and is relaxed for each configuration, see Figure 1. In this paper, L is referred to as the pitch of the strand. For the infinitely wide sheet, the perpendicular distances between the strands are also relaxed. For sampling the k-space,[19] we use two k-points in each direction of the sheet and one k-point in the direction perpendicular to the sheet, which is sufficient for this system. We have calculated the bond energy for a pair of strands (Figure 1a) and for an infinite sheet (Figure 1b). The bond energies, E^{bond}, are calculated relative to the energy of the relaxed single strands for both the pair of strands and for the infinite sheet, see the captions in Figure 1. The pair is the narrowest β-sheet, and the infinite sheet is the broadest sheet possible. Any cooperativity should show up when we compare the bond energy of these two extremes.

In the last section of the paper, we investigate the possible effects from twisting on the cooperativity. We perform simulations on terminated molecules applying the same simulation tools as used for the periodic model. The supercell is so large that the interactions between molecules in neighboring cells are negligible; hence, the terminated molecules are totally free to optimize their structures, see Figure 5. The binding energies for two- and three-strand sheets are calculated, for twisted and nontwisted structures, see the table in Figure 5.

Results and Discussion

We find cooperative effects in all the cases studied (see Figure 1). For the parallel sheets, the additional bonding is 7−11 kJ/mol, which is of the same order of magnitude as the difference in the hydrogen bond strength for different amino acids. Of the three amino acids studied here, the cooperative effect is strongest for Ala. To identify the origin of the effect, we therefore consider parallel strands of Ala in more detail.

First, we investigate if there is an electronic effect as discussed for small molecules in a hydrogen-bonded network and proposed previously for β-sheets.[8,9] We do that by performing calculations where we allow the electrons to relax (polarize) but keep the ion positions fixed, such that no elastic effects are included. If we calculate the energy cost of splitting the infinite sheet rigidly into pairs and compare that energy to the energy cost for splitting the pairs rigidly into single strands, we find that the two bond energies differ by only 2 kJ/mol for both parallel and antiparallel structures. We therefore conclude that electronic effects only contribute slightly to the cooperative effect for the systems considered here. These results agree with earlier results for terminated glycine polypeptides by Zhao et al.,[12] where the effects of relaxing the polypeptide structures were also not included.

Most of the cooperativity that we find is related to structural effects. The key to understanding how this works is to realize that the interaction between any two strands of polypeptides is a strong function of the pitch, L, (see Figure 1). In the present work, the pitch is chosen as the central parameter in the analysis because it is controllable in the simulations without introducing additional constraints, but other structural parameters could have been used such as the local intrastrand distance between carboxyl and amino groups. The energy gained by forming two peptide hydrogen bonds between two strands, $E^{H-bond}(L) = E_{pair}(L) - 2E_{single}(L) = E_2(L) - 2E_1(L)$, is found for all examined structures to be larger the shorter the pitch, L (see Figure 2). The reason is that the freedom of the dihedral angles is larger for small pitches allowing for a better optimization of the hydrogen bond geometry. This effect tends to contract the pair of strands relative to a single strand, but the effect is opposed by the inherent stiffness of the single strands, as defined by the curvature of $E_1(L)$ (see Figure 2). The bond between two strands, $E^{bond}{}_2$, therefore is a compromise between the strongest hydrogen bonds and the lowest cost in elastic energy of each of the strands.

The two competing contributions to the binding energy directly give rise to a cooperative effect. Imagine first letting two strands interact. To get the best hydrogen bonds, the two

(20) Rossmeisl, J.; Hinnemann, B.; Jacobsen, K. W.; Nørskov, J. K.; Olsen, O. H.; Pedersen, J. T. *J. Chem. Phys.* **2003**, *118*, 9783.
(21) Rossmeisl, J.; Kristensen, I.; Gregensen, M.; Nørskov, J. K.; Jacobsen, K. W. *J. Am. Chem. Soc.* **2003**, *125*, 16383.
(22) Ho, B. K.; Curmi, M. G. *J. Mol. Biol.* **2002**, *317*, 291−308.

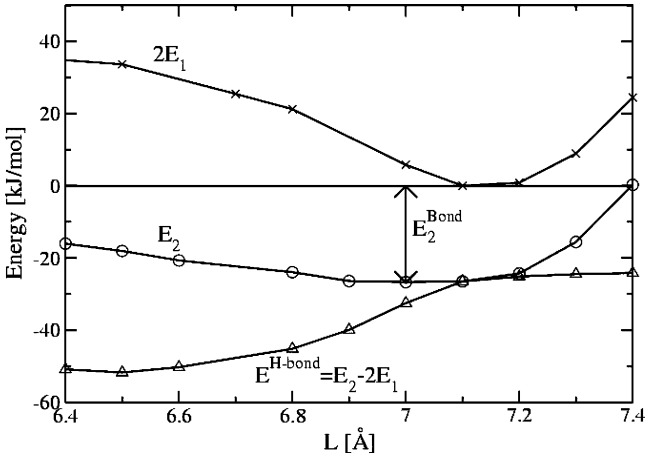


Figure 2. The dependence of the energy of a parallel pair of Ala strands on the pitch, L. $E_1(L)$ is the energy of a single strand relative to the equilibrium energy at $L = 7.2$ Å, $E_2(L)$ is the energy of an interacting pair of strands, relative to the equilibrium energy of two single strands. $E^{\mathrm{H-bond}}(L)$ is defined as the difference $E^{\mathrm{H-bond}}(L) \equiv E_2(L) - 2E_1(L)$, which is the energy gained by forming two hydrogen bonds at a given L. The hydrogen bonding is optimal at $L = 6.5$ Å. The resulting binding energy between the interacting pair of strands, $E^{\mathrm{bond}}{}_2$, is indicated by the arrow.

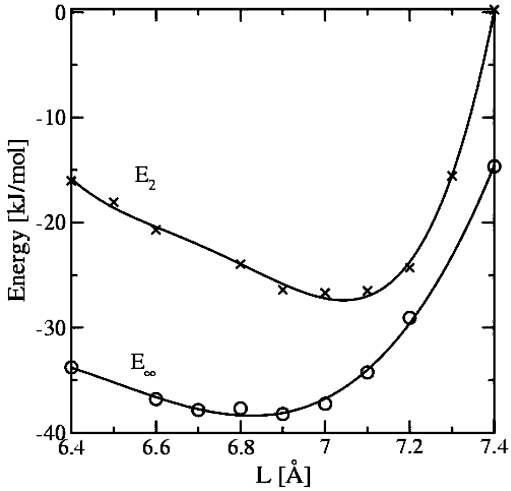


Figure 3. The calculated energies of the two-strand parallel Ala sheet and the infinite parallel Ala sheet as functions of the pitch, L. The full lines are polynomial fits to the calculated points. The minimum energies are $E^{\mathrm{bond}}{}_2$ and $E^{\mathrm{bond}}{}_\infty$, respectively. The minima are shifted from $L = 7.05$ Å for the parallel two-strand sheet to $L = 6.83$ Å for the infinite sheet.

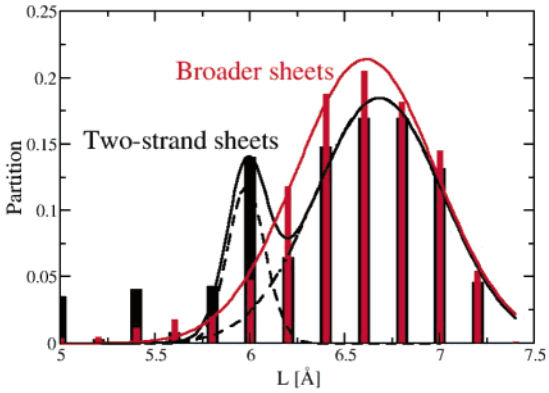


Figure 4. The distribution in the distance $C_\alpha - C_{\alpha+2}$, L, for two-strand sheets (black bars) and all broader sheets (red bars), from a set of protein structures (23). The lines indicate the best fit by Gauss functions. The two-strand sheets show two pronounced peaks, thus two Gaussians are needed (dashed lines). The broader sheets only have one peak. The peak at $L = 6.0$ corresponds to a 2_7-ribbon intrastrand hydrogen bond (24), not relevant in this context. The peaks at longer L show the fingerprint of elastic cooperativity: the two-strand sheets tend to be longer than the broader sheets. The shift is ~0.1 Å, which is in the range predicted by the simulation. The standard deviation on the average is 0.026 Å and 0.006 Å for the two-strand sheets and for the broader sheets, respectively.

strands have to shorten a little, and the net binding energy is weak because it costs energy to shorten the two strands. Now add a third strand. This strand also has to shorten, but the strand to which it bonds is shortened already, so the energy cost is only about half that of the elastic energy needed to form the first bond; thus, a stronger bond results! Three strands have two sets of hydrogen bonds and thus tend to shorten the pitch even further making the bond to a fourth strand even stronger. This trend will continue as the sheet gets broader, but the extra bonding becomes smaller and smaller as the result approaches the limit of an infinite sheet. To put it in a different way: for narrow two-strand sheets, there is only one set of hydrogen bonds but two strands have to be shortened. For a four-strand sheet, there are three sets of bonds to carry the energetically cost of shortening four strands. A cooperative effect thus results.

Our calculations point to a new cooperative mechanism for the bonding in polypeptide sheets. The interaction energies are significant on scale of thermal energies, since the cooperativity is additive in the sense that the size of the effect scales with the number of residues in the strands. Thus, the effect provides a likely explanation of the observed tendencies for cooperative bonding in real β-sheets. The proposed elastic mechanism can be directly confirmed for real proteins. The coupling between the strength of the hydrogen bonds and the pitch, L, results in a shorter pitch for broad sheets than for narrow sheets, as shown in Figure 3. This can be tested against experimental data from the Protein Data Bank. The distance between C_α and $C_{\alpha+2}$ measured in protein structures is denoted L, as this distance is directly comparable to the pitch in the simulation. Figure 4 shows the distribution of L for β-sheets with two strands and with more than two strands. The set of protein structures are from ref 23. Clearly, the main peak for two strands has an average value of L which is ~0.1 Å larger than for the rest. This is the right order of magnitude compared with the predictions of the calculations: We find that Ala strands have a contraction ~0.2 Å, see Figure 3, whereas Val strands have a smaller one ~0.05 Å. The two-strand distribution in Figure 4 has a second, smaller peak at low pitches. We associate this with two-strand antiparallel sheets forming an intrastrand hydrogen bond so that the strands are in a 2_7-ribbon structure. This has previously been observed to cause an extra peak in the distribution of the intrastrand distance between the H_i and O_i, which is a parameter closely related to L.[24]

The antiparallel β-sheets tend to show a smaller cooperative effect than the parallel ones, see the table in Figure 1. The reason is that the tendency for $E^{\mathrm{H-bond}}(L)$ to decrease for small L is weaker for the antiparallel strands because here the hydrogen bonds can become quite strong even without contraction. It has been suggested that cooperativity is crucial for the stability of parallel sheets, as real parallel β-sheets seldom contain less than four strands. Narrow antiparallel sheets are, on the other hand,

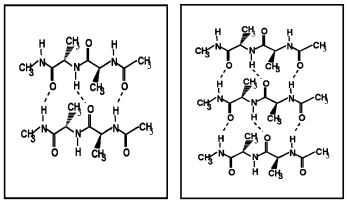
(23) Rost, B.; Sander, C. *J. Mol. Biol.* **1993**, *232*, 584−599.

(24) Shamovsky, I. L.; Ross, G. M.; Riopelle, R. J. *J. Phys. Chem. B* **2000**, *104*, 11296−11307.

often found in native proteins.[25−27] This suggestion is clearly supported by our model.

The structural model we use treats idealized periodic polypeptides and even though the model is considerably more realistic than previous models (with small, terminated polypeptides), there is the possibility that the cooperativity in real β-sheets is also affected by twisting. In native protein structures, β-sheets often show a right-hand twist. By applying periodic boundary conditions, we avoid the effects from the terminating peptide bonds, but on the other hand the boundary conditions constrain the sheets so that no twisting is possible. The twisting is a very soft mode and hence the energy related to this degree of freedom is small.[28] However, twisting has an effect on the dihedral angle and could therefore affect the cooperativity proposed here. To investigate the possible effects of twisting, we have performed simulations on a molecule containing three peptide bonds and terminated with CH_3 groups, see Figure 5. Parallel sheets with one, two, and three strands are relaxed. The dimensions of the unit cell are $16 \times 20 \times 12$ Å^3 and there is corrected dipole moment of the unit cell in the direction of the sheet perpendicular to the direction of the strands, otherwise, the method is the same as for the periodic model.

We find both twisted and nontwisted stable structures. The calculated energy related to twisting is negligible, see Figure 5. Because of the terminations, the binding per peptide bond is clearly stronger than the binding found in the periodic model. However, the size of the cooperativity per peptide bond is conserved. This shows that the constraints from the periodic boundaries do not affect the size of the cooperative effect.



initial twist	E_2^{Bond}	E_3^{Bond}	$\Delta E = E_3^{Bond} - 2E_2^{Bond}$
0°	−60 kJ/mol	−130kJ/mol	−10kJ/mol
20°	−59 kJ/mol	−131kJ/mol	−12kJ/mol

Figure 5. Top: A schematic representation of the two- and three-strand parallel sheets containing terminated polypeptides. Bottom from the left: the initial twist, the binding energy of the two-strand sheet, $E^{bond}{}_2$, and of the three-strand sheet, $E^{bond}{}_3$. The cooperative binding energy is calculated as the difference $\Delta E = E^{bond}{}_3 - 2E^{bond}{}_2$.

Conclusion

In summary, density functional calculations directly show that the hydrogen bond strength in β-sheets increases with the number of strands in the sheet. The calculations allow for a molecular level understanding of the origin of the effect. The mechanism can be directly observed as a contraction of the pitch of β-sheets as the number of strands in the sheet increases. The calculations have one final prediction. They show that the local strength of the cooperative effect depends on the amino acid involved. This suggests that by varying the sequence of amino acids in a β-sheet one can control the bonding properties and thus the ability to aggregate.

Acknowledgment. We acknowledge support from Danish Center for Scientific Computing through grant no. HDW-1101-05. CAMP is sponsored by the Danish National Research Foundation.

JA0490626

(25) Sternberg, M. J. E.; Thornton, J. M. *J. Mol. Biol.* **1976**, *110*, 285−296.
(26) Richandson, J. S. *Nature* **1977**, *268*, 495−500.
(27) Ruczinski, I.; Kooperberg, C.; Bonneau, R.; Baker, D. *Proteins* **2002**, *48*, 85−97.
(28) Emberly, E. G. et al. Flexibility of beta-sheets: Principal-component analysis of database protein structures. cond-mat/0309119 (Sept 2003).