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Biophysics of Structure and Mechanism

Discussion* to I. Primary Reactions of Photoreception and Comparison with Photosynthesis

Reported by R. K. Clayton** (Chairman), P. Carl***, and G. Maaz***

Introduction

What can Investigators of Photosynthesis and Vision Learn from each Other?

R. K. Clayton

One of the aims of this EMBO Workshop was to expose the participants to the viewpoints of investigators in areas that are related, conceptually or methodologically, to vision. One such area of study is photosynthesis (see Chapter 1, ref. 1).

The membrane-bound discs of rod outer segments have their structural counterparts, in photosynthetic tissues, in vesicular bodies bounded by membranes that carry the photosynthetic apparatus. These bodies are the thylakoids of chloroplasts and the variously shaped invaginations and inclusions derived from the cytoplasmic membranes of photosynthetic bacteria. In the photosynthetic membranes, chlorophylls and accessory pigments are bound non-covalently to proteins and lipids. Most of the pigment acts as an antenna to absorb light and to deliver the energy, in the form of singlet excitation quanta, to special sites (reaction centers) that execute the primary photochemistry.

Reaction centers have been isolated as specific pigment-protein complexes from photosynthetic bacteria [2–5] but not from any green plant or algal tissue. There is abundant evidence, however, that green plants and algae contain reaction centers that function in essentially the same way as the bacterial reaction centers. Green plants have two kinds of reaction centers, one coupled to the reduction of pyridine nucleotide and the other to the evolution of oxygen from water. Each species of

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** Division of Biological Sciences, Cornell University, Ithaca, New York 14 853, USA

*** Institut für Neurobiologie der Kernforschungsanlage GmbH, Postfach 1913, D-5170 Jülich, Federal Republic of Germany

photosynthetic bacterium (excluding the blue-green algae or cyanobacteria) has just one kind of reaction center; for several species the reaction center has been purified and characterized in detail. Our comparisons with the visual process can sensibly be restricted to the relatively simple bacterial photosynthesis and indeed to the single species *Rhodopseudomonas sphaeroides*, for which the primary photochemistry has been elucidated in the greatest detail [6–9]. Less complete studies with other species have shown that the essential features of the photochemical mechanism in *R. sphaeroides* are applicable to many other photosynthetic organisms.

Reaction centers from *R. sphaeroides* contain bacteriochlorophyll (Bchl) bacteriopheophytin (the analogue of Bchl in which the central magnesium atom has been replaced by two hydrogen atoms), ubiquinone and iron, all bound to a protein of molecular weight less than 100,000. In the intact membrane this entity is accompanied by the much more abundant "antenna" Bchl and carotenoid pigments. When the Bchl of the reaction center is raised to its lowest excited singlet state, either by direct absorption or by delivery of a quantum from the antenna, a photochemical reaction ensues within 10 ps, in which an electron is transferred from Bchl to bacteriopheophytin. With half-time about 200 ps the electron moves on from bacteriopheophytin to ubiquinone. The resulting photochemical products in the reaction center, oxidized Bchl and reduced ubiquinone, are stable against recombination for more than 20 ms. This degree of stability is enough to ensure that the products react efficiently with secondary electron carriers. The result is a cycle of electron transfer from "primary" ubiquinone to other quinones and cytochromes, and back to the oxidized Bchl. Completion of the cycle restores the components of the reaction center to their "neutral" forms, ready to react again. Oxidations and reductions of some of the carriers in the cycle are attended by the binding or release of protons in such a way that the cycle transports protons across the membrane [10]. Repetition of the light-driven cycle thus generates a proton gradient across the membrane; some of this gradient is translated, by the diffusion of other ions, into a membrane potential. In summary, the energy of light is first converted, in the reaction centers, to the energy of separated positive and negative charge. This is translated into the energy of an electrochemical state of the membrane-bound vesicle, partly a difference in H^+ concentration inside and outside the vesicle, and partly an electric potential across the membrane. These two components of electrochemical energy somehow [11] mediate the phosphorylation of ADP, so that a fraction of the energy of a quantum of light is ultimately stored in an anhydride phosphate bond of ATP.

Cyclic electron transport coupled to ATP formation appears to be the predominant means of storing energy in most photosynthetic bacteria. In green plants a major enterprise is the non-cyclic transfer of electrons from water to pyridine nucleotide, which in turn mediates the reduction of carbon dioxide to carbohydrates and other organic compounds. But this metabolism also uses ATP, and the flow of electrons between the two distinct photosystems of green plants is coupled to proton translocation and ATP formation.

Perhaps the most intriguing link between the mechanisms of photosynthesis and vision is the case of bacteriorhodopsin in the membranes of certain halophilic bacteria [12, 13]. Light absorbed by bacteriorhodopsin leads to the translocation of protons across the membrane and to the formation of ATP. But the suggestion of a "visual" pigment performing an analogue of bacterial photosynthesis is deceptive.

The movement of protons in the purple membranes of halophilic bacteria is probably based on a cycle of states of the bacteriorhodopsin, and this might involve the movement of electrons among different parts of the chromoprotein, but it does not depend on a cycle of peripheral electron carriers as found in the photosynthetic bacteria. Also there is little evidence that proton translocation initiates visual excitation in higher animals.

Although the mechanistic similarities between photosynthesis and vision are tenuous, the practitioners of each field might profit by examining the principal methodologies of the other.

The photochemistry of photosynthesis is oxido-reductive, and the study of photosynthesis has been in large measure the examination of interrelated oxidations and reductions. A change in oxidation state of a compound is often accompanied by a change of energy states associated with electron spin, which can be detected by microwave spectroscopy: both conventional electron spin resonance measurement and the more sophisticated electron-nuclear double resonance technique [14]. In fact, the first biological application of electron spin resonance measurement was the detection of oxidized reaction center chlorophyll in plant tissues [15].

The components of oxidation-reduction reactions can be characterized by redox potentiometry; one simply examines the reactivity or the state of a component while varying the ambient redox potential [16, 17].

These techniques are not likely to have great utility in studying vision, but a few experimental adventures, such as examining the effects of oxidants and reductants on visual excitation and adaptation, might prove unexpectedly stimulating.

The greatest single tool in recent decades of photosynthesis research has been sensitive differential absorption spectrometry. Most of the components of the photochemical process and associated electron transfer events have been detected, quantified, and described in terms of interactions and kinetics by measuring changes of optical absorption that attend their reactions. In intact tissues and membrane fragments, the reactive components make up a small fraction of the total light-absorbing material; they are overshadowed one hundred fold or more by the antenna pigments. Small fractional changes of absorbance must be measured, and this fact has stimulated the greatest possible improvement in the sensitivity (signal : noise ratio) of measurement. This problem was relieved somewhat when purified reaction centers became available, but most of the interesting events that occur after the photochemistry take place outside the reaction centers and must be studied in the complete, intact tissue. More recently, reaction centers have been especially fruitful objects of study by the newly developed rapid techniques of absorption spectrometry, with time resolved to a few ps or less.

While optical absorption has been a major parameter in the study of vision, its application at the forefront of technical refinement has tended in the past to lag a little behind such applications in photosynthesis. This gap now appears to have been closed in many laboratories.

Resonance Raman spectroscopy, on the other hand, has been exploited with good effect in vision and negligibly in photosynthesis. This is undoubtedly related to the fact that photosynthetic tissues emit considerable fluorescence, which interferes with measurements of inelastic light scattering. The use of this technique will surely become greater in the study of photosynthesis, especially in wavelength regions

where fluorescence is not a problem, but the complementary technique of vibrationally resolved fluorescence spectroscopy may find better application in photosynthesis.

The measurement of chlorophyll fluorescence has been of great help in photosynthesis research; the intensity of fluorescence is an indicator of the population of molecules in the excited singlet state. Variations of the intensity of fluorescence, both in antennas and reaction centers, have given information about mechanisms and patterns of energy transfer and about rates of photochemical utilization by the reaction centers. Perhaps the recently discovered fluorescence of bacteriorhodopsin [18] will lead to new information about the photochemistry of visual pigments.

A major difference between research in vision and in photosynthesis has to do with the difficulty or ease of handling the biological material. Many of the most definitive experiments in vision have required exquisite micromanipulative techniques, whereas most of photosynthesis research does not require the development of such special skills. Very few workers in photosynthesis would attempt to insert microelectrodes into photosynthetic organelles; many are fully developed biochemists with a broad range of expertise in biochemical fractionations and the use of special chemicals such as ionophores that affect membranes and their components in specific ways. Conversely many researchers in vision, preoccupied with the technical challenges of manipulating single cells, have not found the time to acquire a full command of biochemical techniques. Here is a case where each side could profit from the experience of the other.

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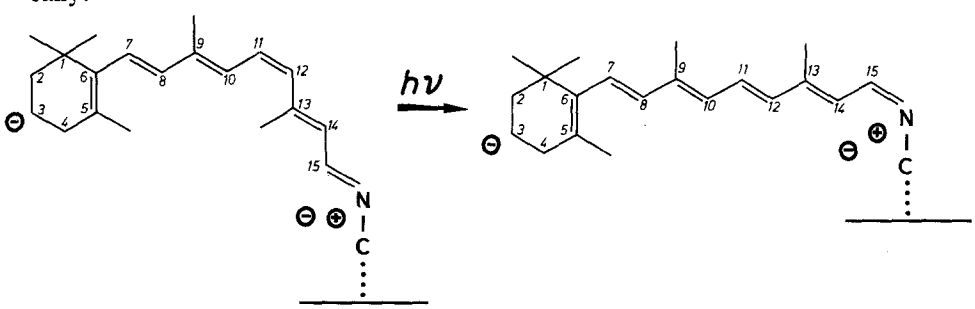
References

1. Clayton, R. K.: Light and living matter, vol. 2. New York: McGraw-Hill 1971
2. Clayton, R. K.: Biochim. biophys. Acta (Amst.) **75,** 312 (1963)
3. Reed, D. W., Clayton, R. K.: Biochem. biophys. Res. Commun. **30,** 471 (1968)
4. Gingras, G., Jolchine, G.: In: Progress in photosynthesis research. Proc. Int. Congr. of Photosynthesis Research, Freudenstadt, 1968, vol. 1, (ed. H. Metzner), p. 209. Tübingen: Int. Union of Biol. Sci. 1969
5. Clayton, R. K., Wang, R. T.: In: Methods in enzymology, vol. 23 (eds. S. P. Colowick, N. O. Kaplan), p. 696. New York: Academic Press 1971
6. Parson, W. W., Clayton, R. K., Cogdell, R. J.: Biochim. biophys. Acta (Amst.) **387,** 265 (1975)
7. Kaufmann, K. J., Dutton, P. L., Nezel, T. L., Leigh, J. S., Rentzepis, P. M.: Science **188,** 1301 (1975)
8. Rockley, M. G., Windsor, M. W., Cogdell, R. J., Parson, W. W.: Proc. nat. Acad. Sci. (Wash.) **72,** 2251 (1975)
9. Fajer, J., Brune, D. C., David, M. S., Forman, A., Spaulding, L. D.: Proc. nat. Acad. Sci. (Wash.) **72,** 4956 (1975)
10. Petty, K. M., Dutton, P. L.: Arch. Biochem. Biophys. **172,** 335 (1976)
11. Greville, G. D.: Curr. Top. Bioenergetics **3,** 1 (1969)
12. Oesterhelt, D., Stoeckenius, W.: Proc. nat. Acad. Sci. (Wash.) **70,** 2853 (1973)
13. Danon, A., Stoeckenius, W.: Proc. nat. Acad. Sci. (Wash.) **71,** 1234 (1974)
14. Feher, G., Hoff, A. J., Isaacson, R. A., Ackerson, L. C.: Ann. N.Y. Acad. Sci. **244,** 239 (1975)

15. Commoner, B., Heise, J. J., Townsend, J.: Proc. nat. Acad. Sci. (Wash.) **42,** 710 (1956)
16. Kuntz, I. D., Loach, P. A., Calvin, M.: Biophys. J. **4,** 227 (1964)
17. Goedheer, J. C.: Brookhaven Symp. Biol. **11,** 325 (1959)
18. Lewis, A., Spoonhower, J., Perreault, G.: Nature (Lond.) **260,** 675 (1976)

R. K. Clayton, Ithaca, N.Y.: The questions are: What is bathorhodopsin?
How does bathorhodopsin differ from rhodopsin?
What is the primary photochemistry?
What is the photochemical product?

T. G. Ebrey, Urbana, Ill.: The classic model for the primary photochemistry of rhodopsin is a cis-trans isomerization about the 11–12 double bond. This does not imply that after isomerization all bonds are trans. There could still be twisting about single bonds and possibly residual twisting about the 11–12 bond. Schematically:



This change
– is photoreversible,
– leaves the Schiff base protonated,
– leads to a red shift of bathorhodopsin compared to rhodopsin and
– could leave bathorhodopsin with more free energy than rhodopsin.
Isorhodopsin could go to a similar or identical photoproduct.

K. Schulten, Göttingen, Fed. Rep. Germany: The primary photochemistry of the visual chromophore is determined by the excited state potential surface. However, which excited state is responsible for the reaction to bathorhodopsin? As the reaction proceeds within a few picoseconds a triplet state appears to be an unlikely candidate. The orthodox theory of polyene spectra assignes a single excited state to the main absorption band of retinal in correspondence to the optically active ^1B_u state in polyenes which is assumed to be the only singlet excitation in the low energy regime. However, Brian Kohler in his lecture presents evidence for the existence of a second low-lying singlet excitation in polyenes which M. Karplus and I assigned on the basis of a many-electron-theory to a two-electron excitation of ^1A_g symmetry. Such '^1A_g' state is predicted on theoretical grounds to exist in addition to the '^1B_u' state also in retinal and its protonated and unprotonated Schiff bases. The relative position of the two states in retinal depends on the geometry of the chromophore and on the strength of electric fields along the π-electron system. In particular, protonation of the Schiff base retinal in the opsin moiety, bond length relaxation and rota-

tion can induce a crossing of the '^1A_g' and the '^1B_u' states so that both states may govern the primary photoreaction of rhodopsin. Of great importance is then the theoretical prediction of an energy minimum of the '^1A_g' potential surface at the 90° position of the 11–12 bond angle. This is in agreement with the 'classical' model which Tom Ebrey just presented in that upon light excitation the protonated Schiff base of retinal is forced to twist out of plane around the 11–12 bond to a 90° angle where it may then descend to the bathorhodopsin ground state potential surface presumably with a minimum near the all-trans configuration.

J. Lugtenburg, Leiden, The Netherlands: The photochemical reaction from rhodopsin to bathorhodopsin is extremely fast, it is even efficient at low temperatures. Upon irradiation rhodopsin, isorhodopsin I and isorhodopsin II all go to the same bathopigment. Isorhodopsin I and II are isomers of rhodopsin; they can be obtained by reacting opsin with 9-cis-retinal and 9–13-dicis-retinal resp. Irradiation of bathorhodopsin gives rhodopsin and isorhodopsin I.

Retinals (and their Schiff bases) can occur in different conformations reached by rotation around the single bonds (these barriers are low). 11-cis-retinal, 9-cis-, 13-cis- and all trans retinal etc. differ in configuration around double bonds and are of course not easily interconvertible. 11-cis-retinal in crystalline state occurs in a 6-s-cis-12-s-cis conformation with dihedral angles of 40° around these single bonds. In solution both 6-s-cis-12-s-cis conformations and 6-s-cis-12-s-trans conformations occur; their energies are comparable.

If we look only at the 6-s-cis-12-s-cis forms of 11-cis retinal, we see that there are two pairs of mirror images. We assume that in rhodopsin only one of the forms is present, such that both 5 CH_3 group and the C_{14}, C_{15} and N atom part are situated at the same face of the central triene part.

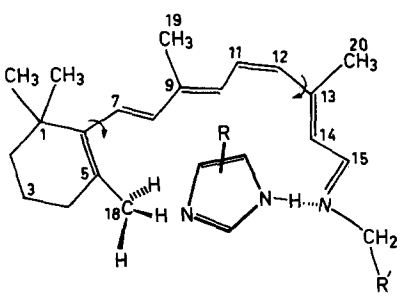


Fig. 1. Representation of the chromophoric group in rhodopsin. The imidazole residue is the ambident group mentioned. The arrows indicate a dihedral angle of 40°

The 5-CH_3 and Schiff base nitrogen are situated such that an ambident group like imidazole, carbonamide, carboxylic acid etc. fits between these parts of the retinylidene group. Upon excitation a hydrogen atom shifts from the 5-CH_3 to the imidazole nitrogen, the other hydrogen shifts from imidazole to the nitrogen in the chain. This changes the position of double and single bonds, we get a hexaene-amine tautomer of the original structure, the position of the atoms and the overall shape of the molecule remain almost the same. In this way no big motions of atoms and changes in shape occur during the photochemical step (Fig. 2 and 3).

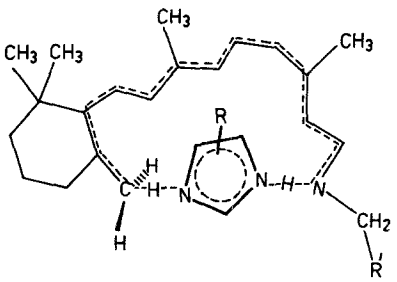


Fig. 2. Representation of the excited state of rhodopsin

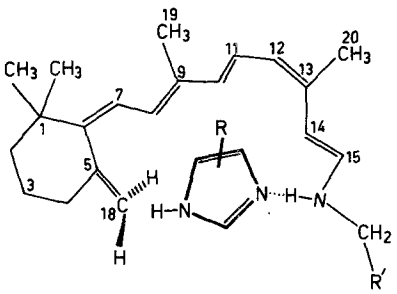


Fig. 3. Representation of the 9-s-trans-11-s-cis conformer of the chromophore in bathorhodopsin

If you start with isorhodopsin I (derived from 9-cis retinal) one can find a 6-s-cis-12-s-cis conformation that fits almost as well an ambident group as discussed for the rhodopsin case. After excitation (and hydrogen shift) one gets an hexaene-amine with the same primary structure as the one obtained in the rhodopsin case. They differ only in conformation around the 9 and 11 single bonds. In the former the hexaene-amine is in the 9-s-trans-11-s-cis conformation and in the latter in the 9-s-cis-11-s-trans conformation. Both forms have comparable energies.

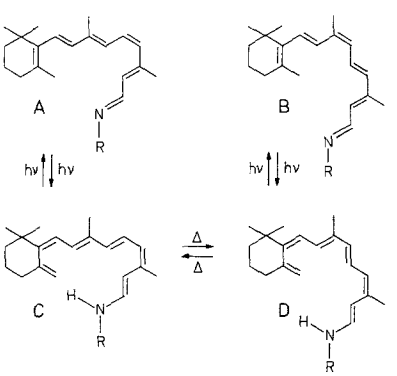


Fig. 4. Planar representation of the chromophore in rhodopsin (*A*) and isorhodopsin (*B*). The chromophore in bathorhodopsin is the equilibrium between the 9-s-trans-11-s-cis (*C*) and the 9-s-cis-11-s-trans (*D*) conformations of the hexaene-amine

We propose that in bathorhodopsin the 9-s-trans-11-s-cis and 9-s-cis-11-s-trans forms are in dynamic equilibrium. (Rotations around single bonds in polyenes have very low barriers e.g. rotation around the single bond in s-cis-butadiene is rapid at about 20 K.)

For isorhodopsin II (9—13-dicis) a conformation of the retinylidene part can be found that will fit the active site in the same fashion as described for both rhodopsin and isorhodopsin I. A hydrogen shift gives the hexaene-amine in the 9-s-cis-11-s-trans-13-s-cis-15-s-cis form. This form is less stable than the 9-s-cis-11-s-trans and 9-s-trans-11-s-cis forms.

The 9-s-cis-11-s-trans-13-s-cis-15-s-cis goes by rotation around the 13 and 15 single bonds into bathorhodopsin, its contribution in the dynamic equilibrium is negligible.

For 13-cis and all trans retinal conformations analogous in shape as described for 9-cis, 11-cis and 9—13-dicis retinal can not be found.

R. K. Clayton: An argument for the cis-trans isomerization is the reversibility of the photochemistry.

J. Lugtenburg: There are very few photochemical reactions where you have trans to cis isomerization at low temperatures and in almost all cases they are frozen out already at much higher temperatures (about −100° C). The protonated Schiff bases have a small trans to cis isomerization quantum yield, which is temperature dependent.

B. Honig, Jerusalem, Israel: There are a number of observations which contradict the model of Lugtenburg. First there are dihydrocompounds, which have no double bonds in the ring, that are known to be photobleached and to isomerize. Such a pigment can isomerize under the influence of light without using its C_5 methyl group. A more conclusive proof was provided by Al Kropf who showed that rhodopsin with 5 desmethyl as chromophore has a bathorhodopsin. Thus you get normal photochemical behaviour without having a methyl group proving that it cannot be an important photochemical determinant. I would like to emphasize that Lugtenburg is forced to assume thermally activated geometry changes at 77 K and in 6 ps to explain that rhodopsin and isorhodopsin have a common intermediate. Thus, the basis of his opposition to photochemical geometry changes is difficult to understand.

J. Lugtenburg: The photochemical step occurs within 6 ps, the conformational equilibrium takes place on a longer time scale.

B. Honig: There isn't any observation of any change at all in the spectrum of bathorhodopsin as a function of time.

J. Lugtenburg: If the spectra are very close together it's quite difficult to observe.

B. Honig: Here again you have to add special assumptions to keep the model alive. In this case you need to have identical spectra.

J. Lugtenburg: Almost identical.

B. Honig: A fourth argument against the model is that in resonance Raman measurements the C=N vibration in bathorhodopsin and rhodopsin appear to be the same. In the Lugtenburg model there is a C—N single bond in bathorhodopsin. Even giving this bond special properties, (themselves unlikely) can hardly explain that the vibration doesn't seem to move at all, when a change of 600 cm^{-1} would be qualitatively expected. Another argument is that the Lugtenburg model for bathorhodopsin should absorb in the UV. The reason that protonated Schiff bases in visual pigments absorb in the red is that they are cations and their positive charge is delocalized. In the proposed structure you not only don't have a cation, you have a nice simple polyene and they absorb in the UV. Bathorhodopsin is red shifted and not blue shifted. A final argument is that in this model there is at a later stage a thermal isomerization which goes to all-trans. So you have to add an unusual step along the bleaching sequence where the binding site changes to prefer all-trans. Here you have another assumption required to make things stick together. You *start* with a binding site which prefers 11-cis, you *end* up with a binding site that prefers 11-cis but in the interim there is a binding site that prefers all-trans; yet when it gets all-trans retinal it kicks it out. This simply doesn't make any sense.

J. Lugtenburg: I don't know what the influence of the protein is on the absorption maximum of the chromophoric part in bathorhodopsin, at least the hexaene-amine structure absorbs at higher wavelengths values than an unperturbed retinal Schiff base.

In relation to the 1655 cm^{-1} peak that is attributed to the C=N double bond in this case a protonated C=N double bond, I would like to make the following remarks: This peak is very weak in the Raman spectra. You find 1655 cm^{-1} peaks too in polyenes, pyrroles and eneamines.

The energy level of bathorhodopsin is about 25—30 kcal/mol above the energy level of rhodopsin. The energy level of opsin plus free all-trans retinal is about 10 kcal/mol above rhodopsin: there is enough driving force to convert bathorhodopsin into opsin and all-trans retinal (via intermediates). There is no reason to assume the presence of an all-trans retinylidene binding site in any of these intermediates.

A. Lewis, Ithaca, N. Y.: In the Raman spectrum of a photosteady state mixture of bathorhodopsin, iso- and rhodopsin you see a triplet bond in the low frequency region at low temperatures. These low frequency modes are absent in the spectra of bacteriorhodopsin and in pure photoreceptor rhodopsin of bovine and squid. This indicates that some change is happening in going from rhodopsin to bathorhodopsin. For example in retinal 2 where there is an extra double bond in the ring you see bands in this region whenever you change the planarity relative to the isoprenoid chain or when you perturb this ring. The other thing that happens is that in the high frequency region there are other bands, which are due to bathorhodopsin and are common to all rhodopsins we have studied. Therefore at the moment I want to say that there are two aspects happening in this system. I say that there must be some

sort of rotational motion or isomerization motion in the system. The secondary effect is also happening in bacteriorhodopsin which I believe has no isomerization. The secondary effect could be an electron withdrawal forming what is called a radical cation. I have a removal of charge from this molecule into a protein species. But what we do know is that there is a double bond to the N with a hydrogen and a positive charge. We have to explain the reduction in the C—C stretch and the reduction in the $C-CH_3$ stretch. But where the chain is rotated I do not know.