# RESONANCE RAMAN STUDIES OF STERICALLY HINDERED CYANOMET "STRAPPED" HEMES

## Effects of Ligand Distortion and Base Tension on Iron-Carbon Bond

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ABSTRACT We report resonance Raman studies of the iron-carbon bond stretching vibrations, $\nu$(Fe-CN), in sterically hindered and unhindered heme ($Fe^{III}$)-$CN^-$ complexes. The sterically hindred "strapped hemes" are equipped with a covalently linked 13-, 14-, or 15-atom hydrocarbon chain across one face of the heme; these are called FeSP-13, FeSP-14, and FeSP-15, respectively. These straps would presumably exert a sideway shearing strain to force the linear ligands (e.g., $CN^-$ and CO) to be tilted and/or bent. The shorter the chain length, the weaker the ligand binding affinity because of a greater steric hindrance. This study reveals that the $\nu$(Fe-CN) frequency decreases as the chain length is decreased, in contrast with the CO complexes, where the $\nu$(Fe-CO) frequency increases as the chain length is decreased. For the heme-$CN^-$ complexes (with *N*-methylimidazole as a base), the $\nu$(Fe-CN) frequencies are: heme 5 (unhindered), 451 $cm^{-1}$; FeSP-15, 447 $cm^{-1}$; FeSP-14, 447 $cm^{-1}$; FeSP-13, 445 $cm^{-1}$. For the heme-CO complexes (with *N*-methylimidazole as a base), the $\nu$(Fe-CO) frequencies are: heme 5, 495 $cm^{-1}$; FeSP-15, 509 $cm^{-1}$; FeSP-14, 512 $cm^{-1}$; FeSP-13, 514 $cm^{-1}$ (Yu, N.-T., E. A. Kerr, B. Ward, and C. K. Chang, 1983, *Biochemistry*, 22:4534–4540). We have also studied the cyanide complexes with three different bases (pyridine, *N*-methylimidazole and 1,2-dimethylimidazole), and found that the *trans*-effect of cyanide complex is different from that of CO complexes. The tension on $Fe^{III}$-base bond weakens the $Fe^{III}$-$CN^-$ bond, whereas the tension on $Fe^{II}$-base bond strengthens the $Fe^{II}$-CO bond. The origin of these differences may be attributed to different extents of the $d\pi$(Fe)- $\pi^*$(ligand) back bonding between the $CN^-$ and CO heme complexes. The Fe-C-N bending vibrations in these cyanomet strapped hemes are not resonance-enhanced, although this bending mode has been detected at ~410 $cm^{-1}$ via Soret excitation in cyanomet insect hemoglobins. It is suggested that the orientation of the tilted Fe-C-N unit may be important in determining the overlap between CN and porphyrin $\pi^*$ orbitals, which provide coupling of the Fe-C-N bending mode with the resonant Soret ($\pi$-$\pi^*$) transition.

### INTRODUCTION

The steric hindrance by distal residues in hemoglobin/myoglobin has been proposed (1-4) as an effective mechanism for lowering the binding affinity of certain ligands such as carbon monoxide and cyanide ion, which prefer to bind to the heme iron in a linear and perpendicular fashion (5). The steric hindrance, especially from E7 and E11 residues, may force them to bind to the iron in an unstable tilted and/or bent geometry. To mimic the influence of nonbonding distal interactions on ligand binding, Ward et al. (6) have synthesized three "strapped" hemes that have a covalently linked 13-, 14-, or 15-atom hydrocarbon strap across one face of the heme (called FeSP-13, FeSP-14, and FeSP-15, respectively). Indeed, these straps lower the CO affinity: the shorter the strap, the lower the CO binding affinity because of the greater steric hindrance (6).

Yu et al. (7) have identified the $Fe^{II}$-CO stretching mode in the Soret-excited resonance Raman spectra of carbonmonoxy strapped hemes, and found that the $\nu$($Fe^{II}$-CO) frequency increases as the strap length decreases. The $Fe^{II}$-C-O bending mode has also been detected in strapped hemes, but not in a simple iron porphyrin (heme-5). Thus, it appears that the Fe-C-O distortion is required for the resonance Raman enhancement of the $Fe^{II}$-C-O bending mode in carbonmonoxy hemes. The observation of the $Fe^{II}$-C-O bending mode in the resonance Raman spectra of carbonmonoxy hemoproteins (8–18) may then be taken as evidence for the $Fe^{II}$-C-O distortion. It has been proposed (7) that the $Fe^{II}$-C-O distortion (primarily tilting with small bending) increases the overlap between CO and porphyrin $\pi^*$ orbitals, which provide direct coupling of the bending mode with the resonant Soret ($\pi$ - $\pi^*$) transition. The metal-ligand stretching and bending modes have also been detected (via Soret excitation) in $Fe^{III}$-NO (19), $Mn^{II}$-NO (20), and $Ru^{II}$-CO (21), which are isoelectronic with the $Fe^{II}$-CO moiety and thus presumably linear and perpendicular to the heme plane in the absence of steric

hindrance (5). In all these systems, however, there is a strong $\pi$-back bonding from metal $d\pi$ to ligand $\pi^*$. Spiro and co-workers (20, 21) have suggested that the enhancement of $Mn^{II}$-N-O and $Ru^{II}$-C-O bending modes in the absence of ligand distortion may be associated with the increased back donation.

In this paper, we report resonance Raman studies of cyanomet heme-5 and strapped hemes, which exhibit predominantly $\sigma$-bonding interactions between iron ($Fe^{III}$) and cyanide ($CN^-$). We demonstrated the resonance Raman enhancement of $Fe^{III}$-$CN^-$ stretching mode in these complexes via Soret excitation. In contrast with the $\nu(Fe^{II}$-CO) stretching mode, we found that the $\nu(Fe^{III}$-$CN^-)$ stretching frequency decreases as the strap length decreases. In other words, the weaker the $CN^-$ binding, the weaker the iron-carbon ($CN^-$) bond. Previously, Yu et al. (7, 22) found that the weaker the CO affinity, the stronger the iron-carbon (CO) bond. Furthermore, we have studied the cyanide complexes with three different bases (pyridine, *N*-methylimidazole, and 1,2-dimethylimidazole). In comparing the $\nu(Fe^{III}$-$CN^-)$ frequencies for the *N*-MeIm and 1,2-$Me_2$Im complexes, we noted that the trans-effect of cyanide complexes is different from that of CO complexes. The tension on $Fe^{III}$-base bond weakens the $Fe^{III}$-CN bond, whereas the tension on $Fe^{II}$-base bond strengthens the $Fe^{II}$-CO bond (22). This may be related to the fact that the $d_\pi$-$\pi^*$ back-bonding is much stronger in the CO hemes than in the $CN^-$ hemes (23).

The Fe-C-N distortion in cyanomet strapped hemes is apparently not sufficient to cause the enhancement of the Fe-C-N bending mode in the Raman spectra. We have not been able to identify this bending mode in all the model complexes examined so far. However, the Fe-C-N bending vibrations in cyanomet insect hemoglobin *Chironomus thummi thummi* III (24) and deuteroheme-substituted CTT IV (25) have been observed at ~410 $cm^{-1}$ via Soret excitation. The Fe-C-N distortion in insect hemoglobins, as well as in strapped hemes, is primarily tilting with little bending because of the Fe-CN stretching frequencies for the $^{13}C^{14}N$ and $^{12}C^{15}N$ isotopes are identical. Thus, additional factors (e.g., orientation of the tilted Fe-C-N unit) may be involved in determining the magnitude of the Franck-condon factor in Albrecht's A-term (totally symmetric scattering) (26).

## EXPERIMENTAL METHODS

The strapped hemes and heme-5 used in this work were synthesized by the method of Ward et al. (6). The cyanide ion was made soluble in benzene by its complex formation with tetrabutylammonium ion. The tetrabutylammonium cyanide was parepared by exchange reaction between $(C_4H_9)_4N^+Br^-$and KCN. A solution containing 4.8 mg of $(C_4H_9)_4N^+Br^-$, 10 ml of benzene, and 0.67 mg of KCN was stirred vigorously for 2 h; solid KBr was then removed by centrifugation.

Preparation of heme (*N*-MeIm)($CN^-$) complexes: 0.5 ml of *N*-MeIm solution (5 mM in benzene) was mixed with 0.1 ml of a heme solution (500 $\mu$M in $CH_2Cl_2$) and 0.2 ml of $(C_4H_9)_4N^+CN^-$ solution (1 mM in benzene); the final volume of the solution was then adjusted to 1.0 ml with benzene.

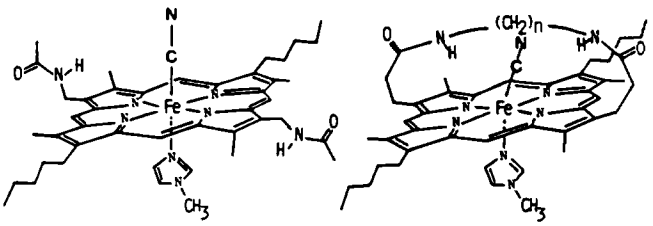


FIGURE 1. Chemical structures of Heme-5, FeSP-13, FeSP-14, and FeSP-15.

Raman spectra were obtained with a highly sensitive multichannel laser Raman system, which has been described in detail previously (27). A krypton-ion laser (model 171-01; Spectra-Physics Inc., Mountain View, CA) was employed to provide the excitation wavelength at 406.7 nm. The laser power at the sample varied between 10 and 30 mW. The sample in the Raman cell was rotated at ~1,000 rpm during the measurements to avoid local heating and possible decomposition. The scattered light was collected at 90° from the incident beam. The entrance slit was set at 100 $\mu$m wide and 0.2 cm high. Fenchone was used to calibrate all spectra, and wavenumbers reported are accurate to $\pm 1$ $cm^{-1}$ for sharp lines and $\pm 2$ $cm^{-1}$ for broad lines.

## RESULTS

The chemical structures of heme-5, FeSP-13, FeSP-14, and FeSP-15 (with *N*-MeIm and cyanide as axial ligands) are shown in Fig. 1. Fig. 2 displays resonance Raman spectra of $Fe^{III}$SP-15 (*N*-MeIm)$CN^-$ in the 200–700 $cm^{-1}$ region with different cyanide isotopes. The only isotope-sensitive line appears at 447 $cm^{-1}$ ($^{12}C^{14}N$), which shifts to 443 $cm^{-1}$ ($^{13}C^{14}N$), 443 $cm^{-1}$ ($^{12}C^{15}N$), and 441 $cm^{-1}$

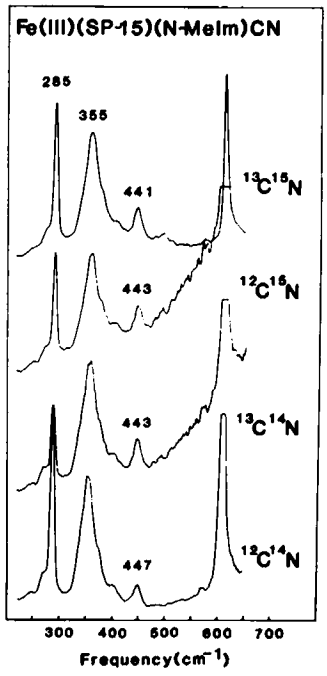


FIGURE 2. Resonance Raman spectra (200–650 $cm^{-1}$) of Fe(III)(SP-15)(*N*-MeIm)CN in benzene/$CH_2Cl_2$ with different cyanide isotopes. Excitation wavelength = 406.7 nm; heme concentration = 50 $\mu$M.

($^{13}C^{15}N$). This line at 447 $cm^{-1}$ is very close to the 453-$cm^{-1}$ line in cyanomet insect hemoglobin CTT III, which has been assigned by Yu et al. (24) as the Fe-CN stretching vibration. The results of our normal coordinate calculations (28) support this assignment. In Fig. 3 we compare resonance Raman spectra of $Fe^{III}X$(*N*-MeIm) $^{12}C^{14}N$ (X = heme-5, SP-15, SP-14, and SP-13) in the 200–700 $cm^{-1}$ region. The Fe-CN stretching mode appears at 451, 447, 447, and 445 $cm^{-1}$ for X = heme-5, SP-15, SP-14, and SP-13, respectively. This clearly shows that as the steric hindrance increases (by decreasing the chain length), the $\nu$(Fe-CN) frequency decreases. Substitution of *N*-MeIm in these complexes by pyridine causes the upshifts of the $\nu$(Fe-CN) frequencies (see Fig. 4) in all four cases. The experimental data with various isotopes for these heme complexes are listed in Table I.

The normal coordinate analysis (28) of the three-body (Fe-C-N) approximation using the Urey-Bradley force field reveals that the Fe-C-N bending mode exhibits a zigzag shift in the order $^{12}C^{14}N \rightarrow {}^{13}C^{14}N \rightarrow {}^{12}C^{15}N \rightarrow {}^{13}C^{15}N$ when the Fe-C-N angle is between 180° and ~160° (see Table II). On the other hand, the stretching frequency of Fe-CN has approximately the same value in both $^{13}C^{14}N$ and $^{12}C^{15}N$ isotopes when the Fe-C-N angle is between 180° and ~175°. It also exhibits zigzag shifts as the Fe-C-N angle becomes <~175°. The isotope-sensitive peak we have found has the same value (443 $cm^{-1}$) in both $^{13}C^{14}N$ and $^{12}C^{15}N$ in $Fe^{III}$ (SP-15) (N-MeIm) $CN^-$ complex. In other cyanide hemes (heme-5, SP-14, and SP-13), we also found that the $\nu$(Fe-CN) frequencies for the $^{13}C^{14}N$ and $^{12}C^{15}N$ isotopes are identical. Thus, it implies that the Fe-C-N linkage is essentially linear in both (*N*-MeIm) and (pyridine) complexes.

The 1,2-dimethylimidazole base is of interest because the 2-methyl group provides steric hindrance (29), causing the tilting and weakening of the Fe-N$\epsilon$(1,2-$Me_2$Im) bond. In other words, this methyl group causes a tension on the Fe-N$\epsilon$(1,2-$Me_2$Im) bond. We have obtained the Fe-CN stretching frequencies for heme-5 and strapped hemes when the base is 1,2-$Me_2$Im (see Table III). It appears that the weaker the base (py > *N*-MeIm > 1,2-$Me_2$Im), the lower the Fe-CN stretching frequencies.

We have taken the differences of the Fe-CN stretching frequencies between the py and the *N*-MeIm hemes (see Table I). The py hemes always have higher Fe-CN stretching frequencies than the corresponding *N*-MeIm hemes. Interestingly enough, there are some regularities among them. In the FeSP-13 hemes the differences in $\nu$(Fe-CN)

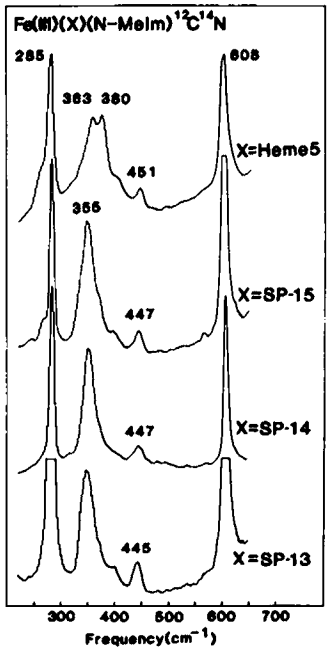


FIGURE 3. Resonance Raman spectra (200–650 $cm^{-1}$) of Fe(III)(X)(*N*-MeIm)$^{12}C^{14}N$, where X = heme-5, SP-15, SP-14, and SP-13. Experimental conditions same as in Fig. 2.

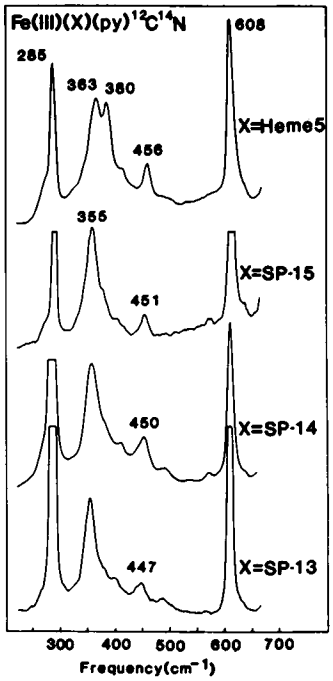


FIGURE 4. Resonance Raman spectra (200–650 $cm^{-1}$) of Fe(III)(X)(py)$^{12}C^{14}N$, where X = heme-5, SP-15, SP-14, and SP-13. Experimental conditions same as in Fig. 2.

TABLE I
ISOTOPE SHIFTS OF FE(III) (X) (Y) CN EXCITED AT 406.7 NM (X = SP-13, SP-14, SP-15, AND HEME 5; Y = N-MeIm AND PY)

| | $^{12}C^{14}N$ | $^{13}C^{14}N$ | $^{12}C^{15}N$ | $^{13}C^{15}N$ |
|---|---|---|---|---|
| Fe(III)SP-13 (*N*-MeIm) CN | 445 | 442 | 441 | 438 |
| (Py) CN | 447 | 444 | 443 | 440 |
| Δ | +2 | +2 | +2 | +2 |
| Fe(III)SP-14 (*N*-MeIm) CN | 447 | 443 | 443 | 440 |
| (Py) CN | 450 | 446 | 446 | 443 |
| Δ | +3 | +3 | +3 | +3 |
| Fe(III)SP-15 (*N*-MeIm) CN | 447 | 443 | 443 | 441 |
| (Py) CN | 451 | 447 | 447 | 444 |
| Δ | +4 | +4 | +4 | +3 |
| Fe(III)Heme 5 (*N*-MeIm) CN | 451 | 448 | 447 | 443 |
| (Py) CN | 456 | 451 | 451 | 447 |
| Δ | +5 | +3 | +4 | +4 |

TABLE II
NORMAL COORDINATE ANALYSIS OF THE THREE-BODY (FE-C-N) SYSTEM

| Angle (< Fe-C-N) | Isotopes | Frequencies | | |
|---|---|---|---|---|
| | | Bending (FeCN) | Stretching (Fe-C) | Stretching (CN) |
| | | | $cm^{-1}$ | |
| 175° | $^{12}C^{14}N$ | 410.1 | 459.2 | 2130.4 |
| | $^{13}C^{14}N$ | 399.2 | 452.9 | 2083.3 |
| | $^{12}C^{15}N$ | 406.3 | 453.4 | 2099.6 |
| | $^{13}C^{15}N$ | 395.4 | 447.3 | 2051.7 |
| 170° | $^{12}C^{14}N$ | 403.1 | 472.2 | 2129.8 |
| | $^{13}C^{14}N$ | 393.7 | 464.2 | 2082.9 |
| | $^{12}C^{15}N$ | 398.6 | 467.1 | 2099.0 |
| | $^{13}C^{15}N$ | 389.4 | 459.2 | 2051.2 |
| 160° | $^{12}C^{14}N$ | 386.4 | 502.9 | 2127.3 |
| | $^{13}C^{14}N$ | 379.3 | 492.0 | 2080.7 |
| | $^{12}C^{15}N$ | 381.1 | 498.8 | 2096.3 |
| | $^{13}C^{15}N$ | 374.1 | 487.9 | 2048.8 |
| 150° | $^{12}C^{14}N$ | 385.5 | 539.2 | 2124.0 |
| | $^{13}C^{14}N$ | 379.7 | 525.7 | 2077.8 |
| | $^{12}C^{15}N$ | 379.4 | 535.9 | 2092.6 |
| | $^{13}C^{15}N$ | 373.7 | 522.5 | 2045.7 |
| 140° | $^{12}C^{14}N$ | 408.9 | 578.0 | 2120.5 |
| | $^{13}C^{14}N$ | 403.9 | 562.1 | 2075.0 |
| | $^{12}C^{15}N$ | 401.6 | 575.6 | 2088.6 |
| | $^{13}C^{15}N$ | 396.8 | 559.6 | 2042.4 |
| 130° | $^{12}C^{14}N$ | 471.2 | 618.5 | 2118.3 |
| | $^{13}C^{14}N$ | 466.5 | 600.1 | 2073.7 |
| | $^{12}C^{15}N$ | 462.0 | 616.8 | 2085.8 |
| | $^{13}C^{15}N$ | 457.6 | 598.4 | 2040.4 |

For more details see reference 28. $K_1$(Fe-C) = 2.03, $K_2$(C-N) = 16.63, H(FeCN) = 0.254 mdyn/Å.

between py and *N*-MeIm complexes are exactly 2 $cm^{-1}$ for all the isotopes. In FeSP-14 and FeSP-15 hemes the differences are 3 and 4 $cm^{-1}$, respectively. In the non-strapped heme-5 complexes, the differences are ~4 $cm^{-1}$. Thus, the difference in $\nu$(Fe-CN) between py and *N*-MeIm complexes decreases with increasing steric hindrance.

## DISCUSSION

The differences between Fe-C(CO) and Fe-C(CN) bonds are manifested in two ways. One is in the *trans*-effect: for the CO complexes the weaker the *trans*-base, the stronger the Fe-C(CO) bond; whereas for the $CN^-$ complexes the weaker the *trans*-base, the weaker the Fe-C(CN) bond. Another is in the effect of ligand distortion on Fe-C stretching frequency: for the CO complexes the shorter the chain length (hence greater the distortion), the higher the Fe-CO stretching frequency; whereas for the $CN^-$ complexes, the shorter the chain length, the lower the Fe-CN stretching frequency. In model heme complexes, the Fe-C(CO) bond length (1.77 Å) (30) is shorter than the Fe-C(CN) bond length (1.908 Å) (31).

TABLE III
FE-CN STRETCHING FREQUENCIES ($CM^{-1}$) OF 1,2-$ME_2$IM HEMES EXCITED AT 406.7 NM

| | $^{12}C^{14}N$ | $^{13}C^{14}N$ | $^{12}C^{15}N$ | $^{13}C^{15}N$ |
|---|---|---|---|---|
| Fe(III)SP-13 (1,2-$Me_2$Im) | 441 | 438 | 436 | 435 |
| Fe(III)SP-14 (1,2-$Me_2$Im) | 445 | 442 | — | 436 |
| Fe(III)SP-15 (1,2-$Me_2$Im) | 446 | — | 444 | 441 |
| Fe(III)Heme-5 (1,2-$Me_2$Im) | 448 | 446 | 445 | 443 |

Resonance Raman enhancement of metal-axial ligand (M-AB) stretching vibrations has been observed in several linear and perpendicular systems such as $Fe^{II}$-CO (7, 22), $Fe^{III}$-$CN^-$ (this work), $Fe^{III}$-NO (19), $Mn^{II}$-NO (20), and $Ru^{II}$-CO (21). In a linear geometry, there is no vibrational coupling between M-AB stretching and M-A-B bending. However, in a bent geometry, the two vibrational modes become significantly coupled. Thus, it is conceivable that the M-A-B bending mode can be resonance-enhanced via its coupling with the M-AB stretching mode. In the CO complexes of FeSP-13 and FeSP-14 (7), the isotope-shift data indicated that the Fe-C-O linkage is essentially linear. However, Yu et al. (7) observed the resonance Raman enhancement of the Fe-C-O bending mode in these CO complexes. They have proposed that in the absence of a significantly bent geometry, the $Fe^{II}$-C-O tilting increases the overlap between CO and porphyrin $\pi^*$ orbitals, which provide direct coupling of the bending mode with the resonant Soret ($\pi - \pi^*$) transition. The mechanism by which the bending mode of a linear and perpendicular M-A-B linkage can be resonance-enhanced has been discussed by Spiro and co-workers (20, 21).

It is of interest to note that the $Fe^{III}$-C-N bending mode of cyanomet CTT III (24) has been detected at ~410 $cm^{-1}$; yet the $Fe^{III}$-C-N linkage is essentially linear as indicated by the identical Fe-CN stretching frequencies for the $^{13}C^{14}N$ and $^{12}C^{15}N$ isotopes at 450 $cm^{-1}$. The $Fe^{III}$-C-N bending mode has also been reported in cyanomet complexes of deuteroheme IX-substituted CTT IV (25) and mesoheme IX-substituted CTT IV (25), where the Fe-C-N linkage are linear. Since the distal steric hindrance is likely to be present in these insect hemoglobins, the primary ligand distortion must be the tilting of the linear Fe-C-N unit off the heme normal. Thus, it appears that the overlap between CN and porphyrin $\pi^*$ orbitals may also provide coupling of the Fe-C-N bending mode with the resonant Soret ($\pi - \pi^*$) transition. The lack of enhancement of the Fe-C-N bending mode in cyanomet strapped heme, where the Fe-C-N unit is also tilted, may be attributed to the improper orientation of the Fe-C-N unit so that the overlap between CN and porphyrin $\pi^*$ orbitals is small.

This work was supported by National Institutes of Health grant GM 18894 and National Science Foundation grant CHE-8210200.

*Received for publication 23 January 1987 and in final form 14 July 1987.*

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