Characterization of Secondary Structure of Neocarzinostatin Apoprotein

Kunihito SAITO,*[a] Yukio SATO,[b] Kiyoto EDO,[a] Yuriko AKIYAMA-MURAI,[a] Yoshio KOIDE,[c] Nakao ISHIDA,[d] and Michinao MIZUGAKI[a]

Department of Pharmaceutical Sciences, Tohoku University Hospital,[a] 1–1 Seiryo-machi, Aoba-ku, Sendai 980, Japan, Pharmaceutical Institute, Tohoku University,[b] Aobayama, Aoba-ku, Sendai 980, Japan, POLA Pharmaceutical R & D Laboratory,[c] 560 Kashio-cho, Totuka-ku, Yokohama 244, Japan, and Tohoku University,[d] 2–1–1 Katahira, Aoba-ku, Sendai 980, Japan. *Received April 13, 1989*

Characteristics of the secondary structure of neocarzinostatin apoprotein (apo-NCS) were examined by various means. Gaussian analysis of the Fourier-transform infrared (FT-IR) curve and curve-fitting of the circular dichroism (CD) spectrum for apo-NCS revealed that this peptide was abundant in β-structures. In the presence of sodium dodecyl sulfate (SDS), the CD bands of NCS originating from phenylalanyl, tyrosyl, and cystinyl residues decreased, indicating a conformational change around the chromophore (NCS-chr). On the other hand, apo-NCS, in the SDS system, showed no change of these bands. We showed that the major parts of the protein moiety consist of β-structures by measurements of the FT-IR and CD spectra of apo-NCS and a prediction of the secondary structure based on the amino acid sequence of the peptide. It seems that properties of the protein may be important to the hydrophobic interaction between NCS-chr and apo-NCS.

Keywords neocarzinostatin; neocarzinostatin-apoprotein; neocarzinostatin-chromophore; chromophore environment, secondary structure; parallel pleated structure

Neocarzinostatin (NCS), an antitumor antibiotic peptide, isolated from a culture filtrate of *Streptomyces carzinostaticus* var. F-41,[1] is composed of a protein moiety (apo-neocarzinostatin (apo-NCS), M_r 11000) and a nonprotein chromophore (neocarzinostatin-chromophore (NCS-chr), M_r 659) in the molar ratio of 1 : 1.[2,3] Apo-NCS is composed of many hydrophilic amino acids. This hydrophilic apo-NCS stabilizes the highly hydrophobic and very labile chromophore against the effects of pH, light and heating, and serves as a carrier for NCS-chr.[4,5] The total chemical structure of NCS-chr has been proposed to be a bicyclo-[7.3.0]dodecadiyne[6] having 2-hydroxy-7-methoxy-5-methyl-1-naphthalenecarboxylic acid[7] and α-D-*N*-methyl-fucosamine[8] moieties. Recently, the absolute configuration of NCS-chr was elucidated[9] (Fig. 1). NCS has been characterized as a complex in which NCS-chr is bound reversibly to the protein component, apo-NCS. NCS-chr, which is extractable from NCS by organic solvent,[2,3,10] is responsible for the biological activities of NCS, such as growth inhibition of bacteria and tumor cells, as well as strand scission of deoxyribonucleic acid (DNA) *in vivo* and *in vitro*.[11–13]

The binding between apo-NCS and NCS-chr is specific and no other proteins are exchangeable for apo-NCS.[13] Previously we observed that the carboxyl-terminal-43-peptide residue, especially near the 83-tryptophan residue, obtained by trypsin digestion of apo-NCS can bind to NCS-chr and retains its antimicrobial activity. We also reported that there was a strong hydrophobic interaction between apo-NCS and NCS-chr as well as ionic interaction.[5,14]

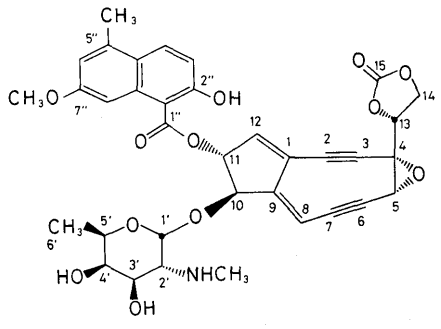

Fig. 1. The Absolute Chemical Structure of NCS-chr

We undertook conformational studies of the peptide backbone of NCS by infrared (IR) spectroscopy and circular dichroism (CD) measurements. Concerning the CD spectrum, contributions from NCS-chr to the near-ultraviolet (UV) CD lead to difficulties in interpretation. However, the CD spectrum of apo-NCS in the far ultraviolet can be interpreted because of the lack of overlapping contributions from NCS-chr. Thus, we will discuss the peptide conformation of apo-NCS based on CD data.

In this study, we examined the characteristics of the secondary structure of apo-NCS to understand the features of the NCS-chr environment and the mode of the reversible binding between apo-NCS and NCS-chr.

Experimental

Chemicals NCS was a gift from Kayaku Antibiotic Research Co. Ltd., Tokyo, Japan. Apo-NCS was purified from NCS according to a modification of the method previously reported.[2] After removal of the NCS-chr by acetic acid extraction from NCS powder, apo-NCS fraction was dissolved in and dialyzed against distilled water at 4 °C. Subsequently, apo-NCS was purified by carboxymethyl-cellulose and Sephadex G-25 column chromatography. The purified apo-NCS showed a single band on polyacrylamide gel isoelectric focusing and sodium dodecyl sulfate polyacrylamide gel electrophoresis. All the other chemicals were purchased from commercial sources and were of analytical grade.

IR Spectra The Fourier-transform infrared (FT-IR) measurements were carried out at room temperature with a JEOL JIR-3500 spectrometer. The spectrum of apo-NCS (1 mg/100 μl) was obtained at 2 cm^{-1} resolution from 1600 to 1700 cm^{-1} (amide I mode) in deuterium oxide solution, and signals of 100 scans were averaged. Triglycine sulfate (TGS) detector and CaF_2 windows were employed on the instrument. The deconvolved spectrum for the prediction of apo-NCS-secondary structure was resolved into Gaussian components by means of a computer program which uses Gauss–Newton iteration.

CD Spectra The CD measurements were carried out at room temperature under constant nitrogen flush, using a JASCO J-400X spectropolarimeter equipped with a data processor. The observations were expressed in terms of molar ellipticity, $[\theta]$, in deg·cm^2·d mol^{-1}. CD spectra were recorded with an apo-NCS concentration of 0.5 mg/ml (in the wavelength region of 260—400 nm) or 0.2 mg/ml (in the wavelength region of 190—270 nm). For measurement of near-UV and far-UV

spectra, 1 and 0.1 cm cells were used, respectively. Apo-NCS secondary structure was analyzed by using the five major secondary structures of proteins presented by Compton and Johnson.[15]

Results

FT-IR Spectrum of Apo-NCS Figure 2 shows an FT-IR spectrum of apo-NCS. Apo-NCS exhibits a strong absorption at 1637 cm^{-1}, amide I band, with two shoulder bands. The deconvolved amide I spectrum can be resolved into five components as illustrated in Fig. 2. The exact positions of these bands are given in Table I along with their total areas (as integrated intensities), which in turn are related to the populations of the corresponding secondary structures. If the amide I bands are of comparable absorptivities (a reasonable approximation), their integrated intensities are a measure of their relative concentrations.[16]

According to Miyazawa and Blout,[17] and Krimm,[18] amide I frequencies at 1634, 1637, and 1685 cm^{-1} are due to β-structure. The absorptions at 1651 and 1658 cm^{-1} indicate α-helix and random coil conformations, respectively. All these results suggest the presence of approximately 60% of β-structure in apo-NCS.

CD Spectrum of Apo-NCS and Estimation of Its Secondary Structure The CD spectrum of apo-NCS showed two negative peaks at about 275 and 214 nm, and two positive peaks at about 226 and 198 nm under physiological conditions (pH 7.4) (Fig. 3). It seems that the CD spectrum of apo-NCS corresponds to that of β-turns (or β-bends).[19]

In the present work, the secondary structure of apo-NCS was estimated by utilizing five basic secondary structures of proteins, α-helix, parallel and antiparallel β-sheets, β-turn, and other (random) structures as presented by Compton and Johnson.[15]

We assumed that the CD spectrum of apo-NCS ($[\theta]_\lambda$) can be expressed by the simplest algorithm combining these features (Eq. 1).

$$[\theta]_\lambda = f_\alpha[\theta]_{\lambda,\alpha} + f_{p\beta}[\theta]_{\lambda,p\beta} + f_{a\beta}[\theta]_{\lambda,a\beta} + f_{\beta t}[\theta]_{\lambda,\beta t} + f_r[\theta]_{\lambda,r} \quad (1)$$

In Eq. 1, $[\theta]_\lambda$ is the observed mean residue ellipticity at a wavelength λ, f_α, $f_{p\beta}$, $f_{a\beta}$, $f_{\beta t}$, and f_r are the fractions of α-helices, parallel and antiparallel β-sheets, β-turns, and random coil structures, respectively; correspondingly, $[\theta]_{\lambda,\alpha}$, $[\theta]_{\lambda,p\beta}$, $[\theta]_{\lambda,a\beta}$, $[\theta]_{\lambda,\beta t}$, and $[\theta]_{\lambda,r}$ are the basic protein CD spectra for these structures.

The curve-fitting analysis for the apo-NCS CD spectrum was made in the wavelength region of 192—244 nm at 2 nm intervals under the conditions of $f_\alpha + f_{p\beta} + f_{a\beta} + f_{\beta t} + f_r = 1$ and all $f \geqq 0$. In Fig. 4, the observed (dotted line) and computed (solid line) CD spectra are illustrated. It is clear that the computed CD well represents the characteristic of the observed CD spectrum of apo-NCS.

From these results, it was indicated that apo-NCS consists of 17% α-helix, 35% parallel β-sheet, 5% antiparallel β-sheet, 28% β-turn, and 15% random coil.

Prediction of Secondary Structure Based on the Sequence For comparison with the results obtained from spectro-

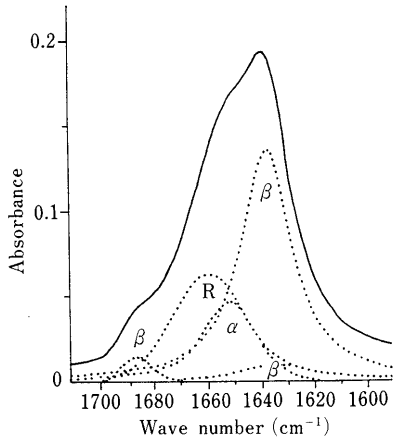

Fig. 2. FT-IR Spectrum of Apo-NCS and Resolution into Gaussian Bands

——, observed amide I band; ------, Gaussian bands. The symbols α,β, and R indicate α-helices, β-structures, and random coil, respectively.

TABLE I. Individual Component Band Areas of Amide I Band of Apo-NCS

ν (cm^{-1})	Area	%[a]
1634	0.5543	5.5
1637	5.1240	50.7
1651	1.7405	17.2
1658	2.4438	24.2
1685	0.2400	2.4

a) Percent of total areas of apo-NCS.

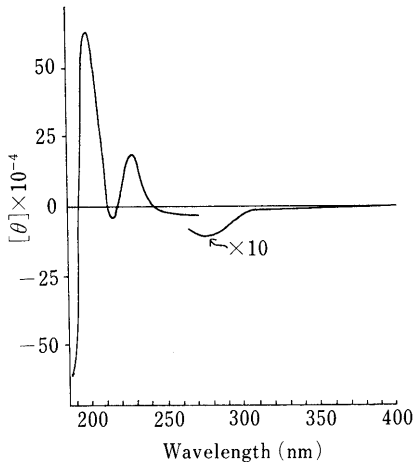

Fig. 3. CD Spectrum of Apo-NCS in 10 mM Phosphate Buffer (pH 7.4)

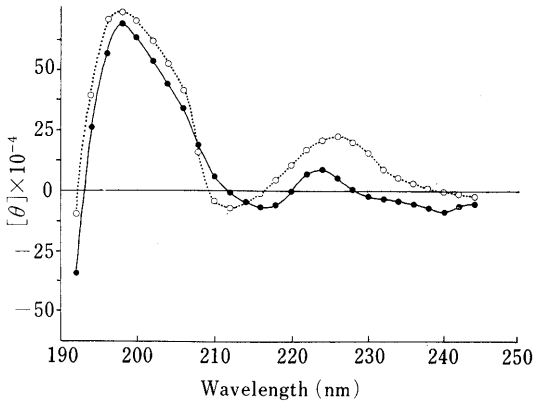

Fig. 4. Comparison of Experimental and Computed CD Spectra of Apo-NCS

—○—, observed spectrum in 10 mM phosphate buffer, pH 7.4; —●—, calculated spectrum.

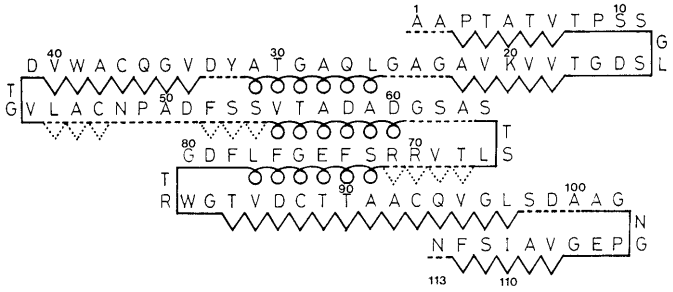

Fig. 5. Schematic Diagram of the Predicted Secondary Structure of Apo-NCS

The prediction is based upon the method given by Chou and Fasman.[20] ϑϑϑ, α-helix; VVV, β-sheet; ⌐⌙⌐, β-turn; ----, random coil. ∿∿∿ represents regions of probable β-sheet.

Table II. Secondary Structure of Apo-NCS Estimated by FT-IR, CD, and Chou and Fasman Methods

	α-Helix (%)	β-Structure (%)		Random coil (%)
FT-IR	17	59	Parallel 6 Antiparallel 53	24
CD	17	68	Parallel 35 Antiparallel 5 Turn 28	15
Chou and Fasman (Genetyx)	16	59	Sheet 31 (40)[a] Turn 28	25 (16)[a]

a) Indicates probable β-sheet.

scopic methods, the secondary structure of apo-NCS was predicted by the probabilistic method given by Chou and Fasman.[20] Previously, we [14] reported the secondary structure of apo-NCS based on its sequence reported by Kuromizu *et al.*[21] In this study, we again estimated the secondary structure of apo-NCS according to the method of Chou and Fasman by the use of Genetyx® (Fig. 5). The result predicted 16% helices, 31% β-resions, 28% β-turns, and 25% random coil (Table II). In Fig. 5, the single letter amino acid sequence is also shown.[21]

Table II presents a summary of the predictions of apo-NCS secondary structure. The overall helical predictions seem to be in good agreement with each other. It appears from Table II that CD analysis overestimated the β-regions compared with the other methods.

Considering the hypothesis of Rose,[22] however, the random coil regions at positions 45—47, 52—54, and 68—72 in Fig. 5 may exist as β-sheets. In this case, the percentage of each form among the total residues would be 16% α-helices, 40% β-sheets, 28% β-turn, and 16% random coil. This result agreed very closely with that obtained by CD analysis. Thus, we can tentatively propose that the secondary structural folding in apo-NCS is as shown schematically in Fig. 5.

Influence of Sodium Dodecyl Sulfate (SDS) on Apo-NCS and NCS Conformations We examined the effects of SDS on the secondary structure of apo-NCS by measuring CD spectra (Fig. 6). As the amount of SDS increased, the positive band at 226 nm decreased in intensity and the negative band at 214 nm increased, while the negative band

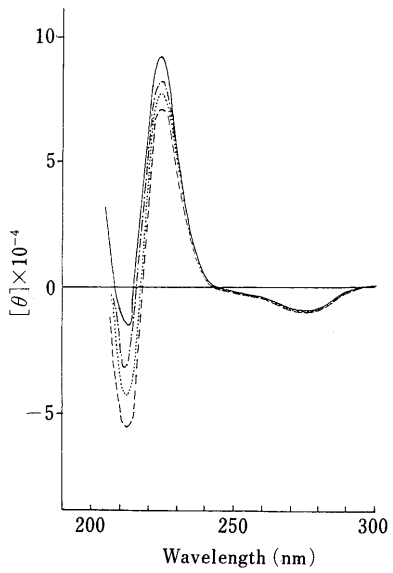

Fig. 6. Change of CD Spectrum of Apo-NCS in the Presence of SDS in 0.1 M Acetate Buffer (pH 5.0)

[apo-NCS] = 2.7×10^{-5} M.
——, apo-NCS alone; —·—. 5 mM SDS; ····, 10 mM SDS; ----, 100 mM SDS.

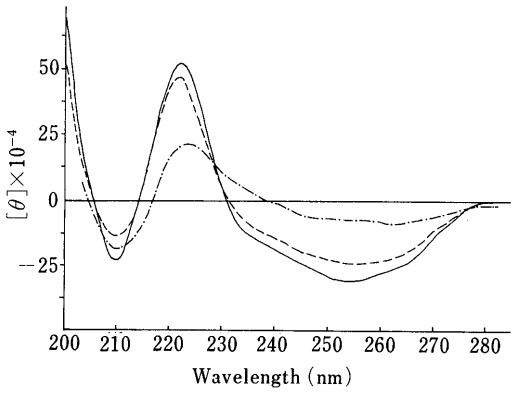

Fig. 7. Change of CD Spectrum of NCS in the Presence of SDS in 0.1 M Acetate Buffer (pH 5.0)

[NCS] = 1×10^{-5} M.
——, NCS alone; ----, 0.2 mM SDS; —·—, 20 mM SDS.

at 273 nm was scarcely influenced. According to the curve-fitting analysis, these spectral changes suggest that the contents of α-helix and parallel β-sheet decreased, while those of antiparallel β-sheet, β-turn, and random coil increased.

The spectral change of the CD of apo-NCS in the presence of SDS was very different from that of NCS (Fig. 7). In the NCS–SDS system, the intensities of all of the three CD bands at 254, 222, and 210 nm progressively decreased with shifts of the CD maxima as the SDS concentration was increased. Since SDS stimulates the release of NCS-chr from NCS, the spectral change in the NCS–SDS system also reflects a conformational change around the NCS-chr binding site. It was observed previously that in the presence of 0.2 mM SDS, no NCS-chr was released from NCS and that more than 80% of NCS-chr was released from NCS at 20 mM SDS.[14] However, the estimation of the secondary structure of NCS was very uncertain, because the influence of the negative band at 253 nm with shoulders at about 265 and 234 nm on the

222 nm band could not be neglected. The wavelengths of these CD bands are different from those given by Maeda *et al.*[23] This discrepancy may arise from the different buffer system used.

Discussion

Environment of NCS-chr NCS has been used in cancer chemotherapy in Japan since 1977.[24] The biological activities of NCS are retained by NCS-chr, which is bound to apo-NCS non-covalently, and is stabilized by the apo-NCS.[25] Moreover, NCS-chr can be reconstituted to NCS with apo-NCS.[26] However, NCS-chr is very labile to light, heat and high pH. Therefore, the NCS-chr binding environment in apo-NCS is important for the biological activities and stability of NCS. Previously, we reported a specific interaction between apo-NCS and NCS-chr.[14] It was postulated that hydrophobic clusters of apo-NCS play an important role in the apo-NCS–NCS-chr complex.

In this study, we examined the secondary structures of apo-NCS by means of FT-IR and CD spectra and prediction according to Chou and Fasman.

Concerning the peptide backbone and aromatic side chain conformations of NCS, Maeda *et al.*[23] reported the presence of antiparallel β-structure on the basis of plane polarized IR spectroscopy and CD results. FT-IR spectroscopy in conjunction with secondary structure prediction methods was used to determine apo-NCS structure. In order to analyze overlapping bands, we used the curve fitting and Fourier deconvolution procedures but not a second derivative method. The result of FT-IR in the present study also showed the presence of β-structures. Further, the absorptions at 1637 and 1685 cm^{-1} may be assigned to antiparallel β-structure.[17,18] This is consistent with the result of plane polarized IR spectroscopy. However, Maeda *et al.*[23] claimed that the possibility of the presence of α-helical structure in NCS is very low. This suggests that the peptide conformation around the NCS-chr in NCS may be antiparallel β-structure, while it may be helical but not random structure in apo-NCS. Such a change of secondary structure would favor the hydrophobic interaction between NCS-chr and apo-NCS.

It is said that turns are the conformations of choice for simultaneously optimizing both backbone-chain compactness and sid-chain clustering.[27] Turns are usually situated at the protein surface. The amino acid sequence is important in determining where a turn exists. β-Turns shown in Fig. 5 are drawn in such a way that the turns are located in the middle positions of the amino acid sequences which have high probability of β-turn formation calculated from the Chou and Fasman statistics.[20] Hence the positions of β-turn in Fig. 5 are tentative. According to Sibanda and Thornton,[28] two-residue loops in β-hairpins are strongly selective for amino-acid type. When the connecting loop residues are designated as L1 and L2, for a type I′ turn, L1 = Asn, Asp, Gly, and L2 = Gly; for a type II′ turn, L1 = Gly and L2 = Ser, Thr or polar residues. The regions in which the amino-acid sequences do not satisfy the requirement for the loop residue are abundant in the amino acids with high preference for the turn.[29] Thus, it seems likely that β-turn can form in the regions shown in Fig. 5. The absence of proline in the β-turn regions in Fig. 5 is not surprising.

The CD spectrum of apo-NCS is different from that of NCS (Figs. 3 and 7). The CD spectrum is atypical both for α-helical and β-form conformation compared with many other water-soluble globular proteins.[30] CD spectroscopy is quite sensitive to the secondary structure of globular proteins. One technique for estimating the secondary structure in proteins has been curve-fitting analysis of a protein CD spectrum assuming a linear relationship between the spectrum of interest and a set of CD spectra corresponding to basic secondary structures.[30] Thus, the basic protein CD spectra for the secondary structures are required to determine the secondary structure of apo-NCS from the CD spectrum.

Apo-NCS contains the following chromophoric amino acids, which contribute to the CD: one tyrosine, five phenylalanines, two cystein disulfides, and two tryptophans. Maeda *et al.*[23] reported that these chromophores, perhaps except for tryptophan residues, contribute to the CD between 230—280 nm. According to Maeda *et al.*, the enhanced CD originating from phenylalanyl chromophores at around 254 nm is due to a polar or charged environment. It appears that the 273 nm band of apo-NCS is also due to these aromatic amino residues. Therefore, the change of this band implies conformational changes around these residues. It was reported that the NCS-chr is released from NCS in the presence of various protein denaturants, such as SDS, urea, and 2-propanol.[14] This indicates that a hydrophobic interaction between NCS-chr and apo-NCS is associated with the formation of the parent compound NCS. In the presence of SDS, the negative band of NCS at 254 nm decreased, whereas the negative band of apo-NCS at 273 nm did not show a further decrease. The difference of CD spectral changes between NCS and apo-NCS reflects the asymmetric nature of these chromophores. That is, in NCS these chromophores are in a buried state, and are exposed by the addition of SDS, resulting in decreases of asymmetric and polar characters.

As shown in Fig. 5, the phenylalanyl, tyrosyl and cystinyl residues are placed in hydrophobic regions. Since the spectral change of NCS in the near-UV region also reflects the NCS-chr release, it can be said that the NCS-chr is located in these hydrophobic regions. Such a consideration is supported by the fact that the C-terminal-43-peptide residue fragment (position 71—113) is necessary for the binding of NCS-chr.[13] From Fig. 5, the C-terminal-43-peptide residue seems to be able to take a very tightly folded conformation. Maeda *et al.*[23] did not interpret the CD in the far-UV region. We have been able to deduce the secondary structure of apo-NCS from the CD spectrum in spite of the fact that the spectrum in the far-UV region is atypical in comparison with many other globular proteins.

The CD spectrum of various conformations in the far-UV arises from peptide chromophores, *i.e.*, peptide bonds rather than amino acid side chains. Thus, the CD spectrum of a protein can be described by a linear combination of reference spectra based on proteins. Chang *et al.*[30] determined the reference CD spectra of four conformations: α-helix, β-form, β-turn, and unordered form. Bolotina *et al.*[31] obtained reference spectra of α-helix, parallel and antiparallel β-structures, β-turn, and unordered form. Recently, Hennessey and Johnson[32] presented five most significant basic CD spectra generated by the eigenvector

method of multicomponent matrix analysis. This method is based on mathematical calculation of orthogonal basic CD spectra from the CD spectra of proteins with known secondary structures. Further, Compton and Johnson[15] constructed the secondary structure spectra for five major secondary structures, α-helix, parallel and antiparallel β-sheets, β-turn, and random structure, from their original inverse CD spectra.

Unfortunately, the CD spectrum of apo-NCS was poorly described by all of these reference spectra except for the spectra of Compton and Johnson.

Although the fit between the experimental curve of apo-NCS and the calculated one based on the reference spectra of Compton and Johnson was not quantitative (Fig. 4), the computed curve qualitatively reflected the characteristics of the observed CD and the estimation for the helix was in fair agreement with those by other prediction methods. The reference spectra cover the wavelength region from 178 to 260 nm, and the lack of consideration in the shorter wavelength region below 190 nm in the present study may account for the poor fit. Thus, the method used in this study seems to be applicable for tentative estimation of the secondary structure of apo-NCS. A good fit between the experimental and calculated curves does not guarantee a correct solution of the secondary structure of a protein, but a poor fit often points up problems in the method of analysis.[33]

From the CD analysis of apo-NCS, it was suggested that apo-NCS contains 35% parallel β-sheet and 5% antiparallel β-sheet. On the other hand, from the Gaussian analysis of FT-IR, 53% antiparallel β-sheet was estimated. From IR measurements, Maeda *et al.*[23] confirmed the presence of antiparallel β-sheet in NCS. The reason for the discrepancy between these estimations is uncertain at present. The most probable explanation seems to be that FT-IR did not fully reflect the fine structure of apo-NCS, especially the peptide conformation around the NCS-chr, because the FT-IR spectrum of NCS in the amide I region was similar to that of apo-NCS (data not shown). In contrast, the CD spectrum of NCS was different from that of apo-NCS, indicating the change of the fine structure.

From the present prediction of the secondary structure of apo-NCS, it is considered that apo-NCS itself possesses a rigid overall conformation, which probably includes α-helix. It seems that α-helix in apo-NCS may change to a packed peptide folding of β-sheet in NCS. Such properties of apo-NCS should be important in connection with the stability of NCS-chr and its biological activities, because a degradation process involves the generation of singlet oxygen.[34] Thus, the conformation of apo-NCS may be very important for binding of NCS-chr.

Similar considerations should apply to apo-proteins of antitumor antibiotics such as macromomycin and auromomycin.[35]

Acknowledgement We are grateful to JEOL for measurements of FT-IR spectra and for valuable discussions.

References

1) N. Ishida, K. Miyazaki, K. Kumagai, and M. Rikimaru, *J. Antibiot., Ser. A*, **18**, 68 (1965).
2) Y. Koide, F. Ishii, K. Hasuda, Y. Koyama, K. Edo, S. Katamine, F. Kitame, and N. Ishida, *J. Antibiot.*, **33**, 342 (1980).
3) M. A. Napier, B. Holmquist, D. J. Strydom, and I. H. Goldberg, *Biochem. Biophys. Res. Commun.*, **89**, 635 (1979).
4) L. S. Kappen and I. H. Goldberg, *Biochemistry*, **19**, 4786 (1980).
5) L. S. Kappen, M. A. Napier, and I. H. Goldberg, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 1970 (1980).
6) K. Edo, M. Mizugaki, Y. Koide, H. Seto, K. Furihata, N. Otake, and N. Ishida, *Tetrahedron Lett.*, **26**, 331 (1985).
7) M. Shibuya, K. Toyooka, and S. Kubota, *Tetrahedron Lett.*, **25**, 1171 (1984).
8) K. Edo, Y. Akiyama, K. Saito, M. Mizugaki, Y. Koide, and N. Ishida, *J. Antibiot.*, **39**, 1615 (1986).
9) A. G. Myers, P. J. Proteau, and T. M. Handel, *J. Am. Chem. Soc.*, **110**, 7212 (1988).
10) H. Suzuki, K. Miura, Y. Kumada, T. Takeuchi, and N. Tanaka, *Biochem. Biophys. Res. Commun.*, **94**, 255 (1980).
11) K. Ohtsuki and N. Ishida, *J. Antibiot.*, **33**, 744 (1980).
12) L. F. Povirk and I. H. Goldberg, *Biochemistry*, **19**, 4773 (1980).
13) K. Edo, K. Saito, Y. Akiyama, M. Mizugaki, Y. Koide, and N. Ishida, *Chem. Pharm. Bull.*, **34**, 5180 (1986).
14) K. Edo, K. Saito, Y. Akiyama-Murai, M. Mizugaki, Y. Koide, and N. Ishida, *J. Antibiot.*, **41**, 554 (1988).
15) L. A. Compton and W. C. Johnson, Jr., *Anal. Biochem.*, **155**, 155 (1986).
16) P. W. Yang, H. H. Mantsch, J. L. R. Arrondo, I. Saint-Girons, Y. Guillou, G. N. Cohen, and O. Bârzu, *Biochemistry*, **26**, 2706 (1987).
17) T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712 (1961).
18) S. Krimm, *J. Mol. Biol.*, **4**, 528 (1962).
19) R. W. Woody, "Peptides, Polypeptides, and Proteins," ed. by E. R. Blout, F. A Bovey, M. Goodman, and N. Lotan, John Wiley and Sons, Inc., New York, 1974, pp. 338—350.
20) P. Y. Chou and G. D. Fasman, *Adv. Enzymol.*, **47**, 45 (1978); *idem*, *Ann. Rev. Biochem.*, **47**, 251 (1978).
21) K. Kuromizu, S. Tsunasawa, H. Maeda, O. Abe, and F. Sakiyama, *Arch. Biochem. Biophys.*, **246**, 199 (1986).
22) G. D. Rose, *Nature* (London), **272**, 586 (1978).
23) H. Maeda, H. Shiraishi, S. Onodera, and N. Ishida, *Int. J. Peptide Protein Res.*, **5** 19 (1973).
24) H. Maeda, *Anticancer Res.*, **1**, 175 (1981).
25) K. Ohtsuki and N. Ishida, *Protein, Nucleic Acid & Enzyme*, **26**, 937 (1981).
26) Y. Koide, A. Ito, F. Ishii, Y. Koyama, K. Edo, and N. Ishida, *J. Antibiot.*, **35**, 766 (1982).
27) G. D. Rose, L. M. Gierasch, and J. A. Smith, *Advan. Protein Chem.*, **37**, 1 (1985).
28) B. L. Sibanda and J. M. Thornton, *Nature* (London), **316**, 170 (1985).
29) J. L. Crawford, W. N. Lipscomb, and C. G. Schellman, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 538 (1973).
30) C. T. Chang, C.-S. C. Wu, and J. T. Yang, *Anal. Biochem.*, **91**, 13 (1978).
31) I. A. Bolotina, V. O. Chekhov, and V. Yu. Lugauskas, *Int. J. Quantum Chem.*, **16**, 819 (1979).
32) J. P. Hennessey, Jr. and W. C. Johnson, Jr., *Biochemistry*, **20**, 1085 (1981).
33) J. T. Yang, C.-S. C. Wu, and H. M. Martinez, *Methods Enzymol.*, **130**, 208 (1986).
34) K. Edo, H. Sato, K. Saito, Y. Akiyama, M. Kato, M. Mizugaki, Y. Koide, and N. Ishida, *J. Antibiot.*, **39**, 535 (1986).
35) N. Miwa, *J. Antibiot.*, **35**, 1553 (1982).