Communications to the Editor

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THE BIOLOGICALLY ACTIVE SITE OF NEOCARZINOSTATIN-CHROMOPHORE

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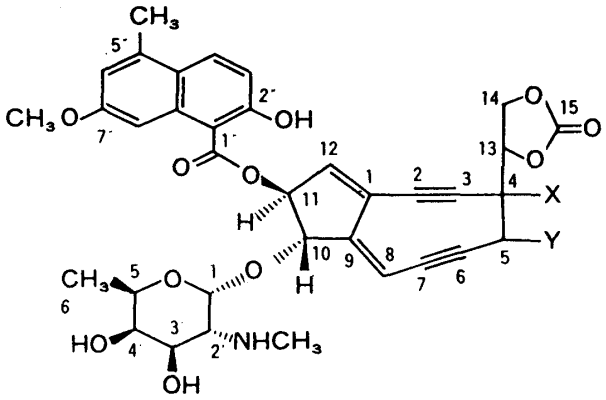
The biologically active site of neocarzinostatin-chromophore (NCS-chr) is determined by the spectral data and biological activities for NCS-chr and related compounds. The bicyclododecadienediyne moiety in the NCS-chr molecule apparently is responsible for its biological activity.

KEYWORDS——neocarzinostatin-chromophore; biologically active site; bicyclododecadienediyne; absorption difference spectrum

Neocarzinostatin-chromophore (NCS-chr) was isolated from the antitumor antibiotic protein drug neocarzinostatin (NCS) by the present writers[1] and others.[2,3] The NCS-chr is tightly bound to its apoprotein (apo-NCS) at a molar ratio of 1:1 and is responsible for the biological activities of NCS, such as its cytotoxic properties against tumor or bacterial cells and DNA strand scission. Apo-NCS, the other component of NCS, stabilizes this chromophore which is very labile in the free form to exposure to ultraviolet (UV) light and heat treatment.[4,5] Recently, we described the chemical structure of NCS-chr as having a novel bicyclo[7,3,0]dodecadienediyne system[6] with 2-hydroxy-7-methoxy-5-methyl-1-naphthalenecarboxylic acid (NA), α-D-N-methylfucosamine (MF), ethylene carbonate (EC) moieties and a highly strained epoxide (SE) on the side chain (Fig. 1). We were recently able to report that, according to the detailed measurements of the extra-weak chemiluminescence of NCS-chrs,[7] the most unstable site in the NCS-chr molecule is the highly strained epoxide. In addition, NCS-chr II (hydrochloride adduct form) retains almost the same biological activity as the native NCS-chr I (epoxide form) (unpublished data). These observations suggest that the biologically active site of NCS-chr is not the epoxide moiety. But it is not clear which functional group(s) is responsible for the biological activity of the NCS-chr molecule. In the course of our NCS-chr research, our interest was focused on the biologically active site of NCS-chr with respect to both its molecular structure and its mode of action.



	X	Y
NCS-chr I	—O—	
NCS-chr II	OH	Cl

Fig. 1. The Chemical Structure of the Neocarzinostatin-Chromophore

In this communication, we describe the spectral data of both the biologically active NCS-chr II and a degradation product (NCS-chr III) and relate these data to the active site of NCS-chr. The activity of NCS-chr related to portions of the molecule is also discussed.

In table I are summarized the elemental analytical data and infrared (IR) spectral data of NCS-chr II and III. In table II are summarized the antimicrobial activities against Micrococcus luteus (M. luteus) and the antitumor activities in rats with Yoshida sarcoma of NCS-chr II, NCS-chr III, the synthetic methyl ester of NA,[8] synthetic D-MF[9] and propylene carbonate. Since NCS-chr III exhibited almost the same elemental analytical data and IR spectral data as NCS-chr II, it retains the native NCS-chr's side chains, NA, MF, and EC. But it has only about 0.1% as much antimicrobial activity as NCS-chr II for the growth inhibition of M. luteus in the presence of apo-NCS in a medium at 10 μg/ ml, and it has less than 1% of the antitumor activity in rats with Yoshida sarcoma. On the other hand, the methyl ester of NA, MF, and propylene carbonate modeled as EC moiety have no antimicrobial activity even at a concentration of 100 μg/ml. These data suggest that it is the other moiety (the bicyclo[7,3,0]dodecadienediyne (BCD) moiety) that constitutes the biologically active site of NCS-chr.

This hypothesis is supported by the absorption spectra of NCS-chr II and NCS-chr III in a methanol solution. It should be noted that the methanol solution of NCS-chr II has characteristic UV absorption at 266, 274, 292, 302, 332 and 352 nm (Fig. 2A). In contrast, NCS-chr III has UV absorption at only 332 and 348 nm with reduced absorption maxima at 266, 274, 292 and 302 nm (Fig. 2B). It is known that NCS-chr II has two chromogens. One is the NA residue and the other is the BCD moiety. NCS-chr II absorbance at 332 and 352 nm can be attributed to NA by comparison with the absorbance of NA (Fig. 2C). The absorbance bands at 274, 292 and 302 nm are due to the BCD moiety because the absorption difference spectrum of NCS-chr II versus the methyl ester of NA exhibits maxima at 278 and 290 nm (Fig. 3A). Furthermore, these UV absorption bands are similar to those of a tri-conjugated ene or yne. Therefore, the characteristic changes in absorption between NCS-chr II and NCS-chr III, which has very weak antimicrobial activity, occurred at 274, 292 and 302 nm (Fig. 2B). This conclusion is strengthened by the absorption difference spectrum of NCS-chr II versus NCS-chr III (Fig. 3B).

The PMR data of NCS-chr III were compared with that of NCS-chr II. Comparison between the PMR data of BCD moiety in NCS-chr II and NCS-chr III is difficult but

Table I. Elemental Analysis and IR Data of NCS-chr II and NCS-chr III

Compound	Formula	Analysis (%) Calcd. (Found) C	H	N	IR (cm^{-1})
NCS-chr II·HCl	$C_{35}H_{34}ClNO_{12}\cdot HCl\cdot 3H_2O$	53.41 (53.44)	5.00 (5.25)	1.57 (1.78)	1811,* 1783
NCS-chr III·HCl**		(51.02)	(4.77)	(1.64)	1811, 1783

* proylene carbonate modeled EC exhibits 1810 cm^{-1}. ** NCS-chr III was obtained by heating or UV irradiation of NCS-chr II in an aqueous solution and purifying by HPLC.

Table II. Biological Activity of NCS, NCS-chr II, NCS-chr III and Related Compounds

Compound	Antimicrobial activity MIC (μg)*	Antitumor activity MED (mg/kg)**
NCS	0.1	0.05
NCS-chr II·HCl	0.006	0.1
NCS-chr III·HCl	6.25	> 10
Methyl ester of NA***	> 100	ND
D-MF***	> 100	ND
Propylene carbonate	> 100	ND

* for the growth inhibition of M. luteus ATCC 9341 in the presence of apo-NCS (10 μg/ml) according to the method of the Japan Society of Chemotheraphy.[10] ** minimum effective dose in rats with Yoshida sarcoma according to Sato's method.[11] *** synthetic samples ND, not determined

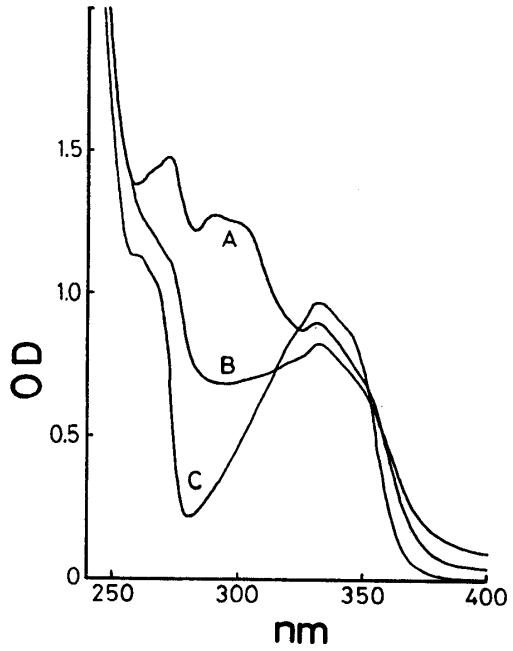


Fig. 2. The Ultraviolet-Visible Absorption Spectra of NCS-chr II, NCS-chr III, and the Methyl Ester of NA

The methanol solution of NCS-chr II, NCS-chr III and the methyl ester of NA, both at 10^{-4} M concentration, were recorded with a Hitachi Model 200-20 spectrophotometer. A: NCS-chr II, B: NCS-chr III, C: methyl ester of NA.

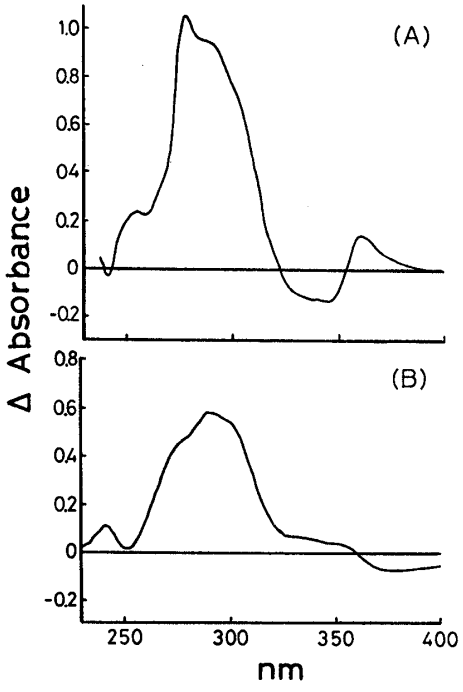


Fig. 3. Ultraviolet-Visible Differential Absorption Spectra of NCS-chr II, NCS-chr III, and the Methyl Ester of NA

The methanol solution of NCS-chr II, NCS-chr III and the methyl ester of NA, both at 10^{-4} M concentration, were recorded with a Hitachi Model 200-20 spectrophotometer. A: NCS-chr II versus the methyl ester of NA, B: NCS-chr II versus NCS-chr III.

it is clear that all resonances of the NA and MF moieties remain relatively unchanged, but the resonances of the four BCD-bound protons in NCS-chr II at 4.95 (BCD H-10), 5.89 (BCD H-8), 6.14 (BCD H-11) and 6.58 (BCD H-12) ppm[6] changed to 5.59, 6.28, 7.48 and 7.72 ppm in the spectrum of NCS-chr III. NCS-chr III may be generated by the ring rearrangement of NCS-chr II's BCD moiety. Detailed structure of NCS-chr III is still under investigation. The above UV and PMR spectral data strongly suggest that the diene-diyne conjugate of the BCD moiety in the NCS-chr molecule is responsible for its biological activity.

Recently, the antitumor activity of the prostaglandin J_2 (PGJ_2) series has been reported by Fukushima et al..[12] They proposed in the course of research on the structure-activity relationship of various PG derivatives that the antitumor active site of PGJ_2 is the cyclopentenone skeleton in its structure. NCS-chr has a 4-hydroxy-2-cyclopenten-1-ylidene moiety in its molecule and the biologically active site of the NCS-chr molecule is located at the 2-cyclopenten-1-ylidene conjugated with two highly strained cyclic acetylenic bonds. The antitumor active site of NCS-chr and PGJ_2 resembles each other in their chemical structures. But a similarity in the action modes of NCS-chr and PGJ_2 derivatives was not clear.

Investigation into the structure of NCS-chr III, the similarity in the action modes of NCS-chr and PGJ_2, and the role of acetylenes in the NCS-chr molecule are now in progress.

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