Biophysical Chemistry 24 (1986) 217–220
Elsevier

BPC 01071

THE MECHANISMS OF INTERACTION BETWEEN FURANOCHROMONES AND DNA

A HETERONUCLEAR OVERHAUSER EFFECT STUDY ON THE KHELLIN-THYMIDINE MODEL SYSTEM

Neri NICCOLAI [a], Lucia BOVALINI [b] and Paola MARTELLI [b]

[a] *Dipartimento di Chimica and* [b] *Istituto di Chimica Biologica, Università di Siena, 53100 Siena, Italy*

Received 21st January 1986
Revised manuscript received 6th May 1986
Accepted 7th May 1986

Key words: Khellin-thymidine complex; Heteronuclear Overhauser effect

The furanochromones khellin and visnagin have been characterised by ^{1}H and ^{13}C mono- and bidimensional NMR spectroscopies. Their strong affinity with DNA was experimentally confirmed by the complete disappearance of the furano-chromones' NMR signals upon additions of DNA. An intermolecular interaction between furanochromones and the thymidyl moieties of DNA, stabilized by the formation of a hydrogen bond between the thymidyl NH hydrogen and the C = O group of khellin or visnagin, is here proposed. This is suggested by the strong donor-acceptor behavior of these two molecular moieties, as pointed out by a selective ^{1}H-^{13}C Overhauser effect study of the khellin-thymidine model system.

1. Introduction

It has been recently shown that ^{1}H-^{13}C selective heteronuclear Overhauser effects (HOE) may be used for delineating intramolecular hydrogen bonding patterns, as well as other conformation features, in biomolecular systems [1–4].

In the present report, the extension of this structural approach for also determining intermolecular hydrogen bonding is discussed by investigating the khellin-thymidine system. The biochemical relevance of the interaction of furanochromones, such as khellin and visnagin, and the thymidyl moieties of DNA is well established: the mechanisms of formation of photoadducts between DNA and furanochromones have been proposed and related to their phototoxicity and genotoxicity [5,6]. Thus, the interaction between khellin and thymidine is here investigated as a model system for the preliminary step of the photoreaction where furanochromones cross-link double-stranded DNA.

2. Materials and methods

Khellin and visnagin (fig. 1) obtained from Angelini and Inverni & Della Beffa Chemical Companies respectively, were dissolved in 99.95% $^{2}H_2O$ and 99.98% DMSO-d_6 together with thymidine or DNA obtained from Sigma. Samples were investigated by using a Varian XL-200 spectrometer operating at 200 and 50.3 MHz for ^{1}H and ^{13}C nuclei. Proton-proton and proton-carbon chemical shift correlation two-dimensional spectra were generated for a complete assignment of ^{1}H and ^{13}C NMR signals of both furanochromones. Selective HOEs were built up by 10 s low-power decoupler preirradiation at the proton frequency of the investigated nuclear spin. A frequency dependence study allowed a better definition of observed HOEs, defined as: $HOE = (M_z - M_0)/M_0$ where M_z and M_0 are the peak intensities measured in on-resonance and off-resonance conditions, respectively. The off-resonance spectrum was obtained by setting the proton decoupler at 12.0 ppm. All measurements were performed at 27°C.

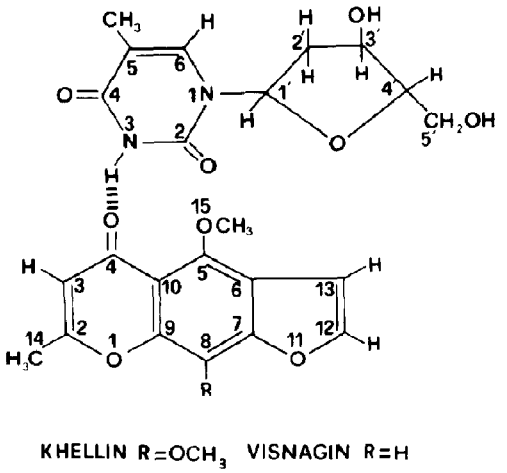

Fig. 1. Khellin, visnagin and thymidine chemical structures. The intermolecular hydrogen bond, proposed in this report, is also shown.

3. Results and discussion

The proton chemical shifts and scalar coupling constants observed for khellin and visnagin are reported in table 1. Additions of a DNA suspension in ^2H_2O to saturated khellin or visnagin solutions determined the complete disappearance of the furanochromone proton signals. A large line broadening, caused by reduced molecular mobility of these molecules, trapped inside the double-stranded biopolymer, can explain this experimental finding. The latter molecular behavior suggests that furanochromones exhibit mechanisms of interaction with DNA which are very similar to those previously found for psoralens [7,8] and in particular that noncovalent interactions between the latter molecules and the pyrimidyl moieties of DNA can form stacking adducts. Then, these noncovalent complexes can be changed into covalent mono-or di-adducts by a photoreaction. It has been shown that steric hindrance, such as a methyl group on the lateral heterocycles, reduces the biological activity of psoralens [9]. Thus, the observed residual activity of khellin in photoreactions has to be ascribed to additional mechanisms of interaction of this molecule with the pyrimidyl moieties of DNA. The khellin-thymidine model system was

Table 1

Furanochromone ^1H chemical shifts and scalar coupling constants

Khellin			Visnagin		
H_n	δ [a] (ppm)	J (Hz)	H_n	δ [a] (ppm)	J (Hz)
12	8.04	$J_{12-13} = 1.5$	12	8.03	$J_{12-13} = 1.5$
13	7.19		13	7.25	
3	6.04		3	6.02	$J_{12-3} = 0.4$
15	4.05		8	7.50	$J_{13-8} = 0.8$
16	3.89		15	4.01	
14	2.32		14	2.27	

[a] Proton chemical shifts from internal TMS of 10 mM furanochromones in DMSO-d_6 solution at 27°C. A ^2H_2O titration of the latter solutions indicated that the chemical shift values in the aqueous solvent are identical except for H_{12} which exhibits a slight upfield shift (0.02 ppm in the DMSO ^2H_2O, 1:1, mixture).

then, studied in order to elucidate the intermolecular interaction process. In particular, the formation of intermolecular hydrogen bonds was investigated. From a preliminary mono- and two-dimensional ^{13}C-NMR study of this molecular system the data reported in table 2 were determined. It is worth noting that the simultaneous presence of the two molecules does not alter significantly the khellin and thymidine ^{13}C chemical shifts. This finding indicates that stacking interactions between khellin and thymidine, if present, have strength and nature similar to those occurring among khellin (or thymidine) molecules or that the existing differences do not yield detectable contributions to the observed NMR parameters under our experimental conditions.

The selective decoupler irradiation in the frequency region of the thymidine NH proton yielded three HOEs, as shown in fig. 2. Two of these are similar and very intense, both arising from short range ($\sim$ 2.0 Å) geminal proton-carbon dipolar interactions. The third HOE clearly indicates that the thymidine NH proton and khellin $C_4 = O$ carbon under our experimental conditions are characterized by a close spatial proximity. Only intermolecular hydrogen bonding between these two molecular moieties can explain the occurrence of this HOE. Furthermore, it should be noted that (i) this interaction involves proton-carbon internuclear distances which are typically

Table 2

^{13}C chemical shifts of khellin and thymidine

Khellin			Thymidine		
C_n	δ [a] (ppm)	$\Delta\delta$ [b]	C_n	δ [a] (ppm)	$\Delta\delta$ [b]
4	176.48	−0.03	4	163.53	0.03
2	164.08	−0.05	2	150.27	0.04
7	148.17	−0.07	6	135.92	0.03
12	146.82	−0.12	5	109.17	0.07
9	146.61	−0.09	4′	87.1	0.1
8	146.43	-0.07	1′	83.67	0.1
5	129.31	−0.06	3′	70.27	0.12
6	118.81	−0.06	5′	61.2	0.11
10	113.11	−0.07	CH_3	12.02	0.13
3	109.88	−0.05			
13	104.98	−0.04			
16	61.67	0.0			
15	61.07	0.0			
14	19.38	+0.05			

[a] ^{13}C chemical shifts from internal TMS of 0.2 M khellin and 0.4 M thymidine solutions in DMSO-d_6 at 27°C.

[b] Chemical shift differences induced by the simultaneous presence of 0.2 M khellin and 0.4 M thymidine in solution.

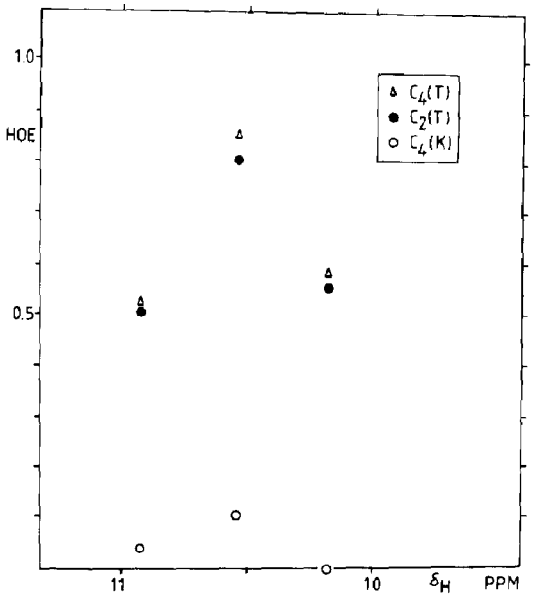

Fig. 2. Frequency dependence of heteronuclear proton-carbon Overhauser effects (HOEs) upon decoupler selective saturation in the chemical shift region of the thymidine NH proton. Thymidine and khellin concentrations in the DMSO solution were 0.2 and 0.4 M, respectively.

larger than 2.5 Å, (ii) Overhauser effects have an r_{ij}^{-6} dependence on the *i-j* dipolar interaction, and (iii) multiple equilibria exist in solution which account for all possible solvent-solute and solute-solute complex formation. It follows that the latter intermolecular hydrogen bond yielding a relatively intense HOE must involve a large fraction of khellin molecules and, hence, this furanochromone exhibits a strong chemical affinity with thymidine.

It can be concluded that proton-carbon selective HOE measurements represent a very promising approach for the simultaneous identification of acceptor-donor moieties of intermolecular hydrogen bonding.

Moreover, it can be proposed that DNA and khellin interact via hydrogen bonding and stacking processes.

One can also propose complexation of khellin with a double-stranded DNA which is stabilized on one side by a stacking interaction between the furanic and thymidyl moieties of the two molecules. On the other side the $C_4=O$ group of khellin, hydrogen bonded to the thymidyl NH proton, favors the intercalation of the furanochromone between adjacent base pairs. In this way a photoreaction can occur yielding an interstrand cross-link.

Acknowledgements

This work was supported by grants from the Italian C.N.R. (Italy/U.K. Cooperative Science Agreement). Thanks are due to Dr. C. Rossi, Dr. M. Mancini and Miss. K. Scrivener for helpful assistance.

References

1 N. Niccolai, C. Rossi, V. Brizzi and W.A. Gibbons, J. Am. Chem. Soc. 106 (1984) 5732.

2 N. Niccolai, C. Rossi, P. Maseagni, P. Neri and W.A. Gibbons, Biophys. Biochem. Res. Commun. 124 (1984) 739.

3 N. Niccolai, A. Sega, M. Scotton and C. Rossi, Gaz. Chim. Ital. 115 (1985) 149.
4 N. Niccolai, C. Rossi, P. Mascagni, V. Brizzi and W.A. Gibbons, J. Chem. Soc. Perkin I (1985) 239.
5 B.F. Abeysekera, Z. Abramowski and G.H.N. Towers, Photochem. Photobiol. 38 (1983) 311.
6 P. Martelli, L. Bovalini, S. Ferri, G.G. Franchi and M. Bari, FEBS Lett. (1986) in the press.
7 K. Straub, D. Kanne, J.E. Hearst and H. Rapoport, J. Am. Chem. Soc. 103 (1981) 2347.
8 D.A. Pearlman, S.R. Holbrook, D.H. Pirkle and S.H. Kim, Science 227 (1985) 1304.
9 F. Dall'Acqua, S. Marciani, L. Ciavatta and G. Rodighiero, Z. Naturforsch 26B (1971) 561.