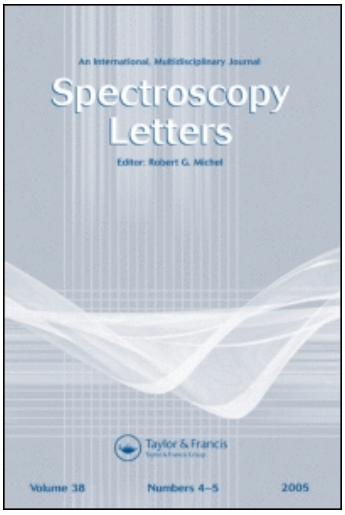

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

NMR STRUCTURAL INVESTIGATION OF THE BIOTIN-AVIDIN COMPLEX: A ^{13}C NMR PARAMAGNETIC RELAXATION STUDY

M. Scarselli[a]; A. Vasco[a]; A. Bonci[a]; M. Rustici[a]; N. Niccolai[a]
[a] Contribution from the Dipartimento di Biologia Molecolare, Università di Siena, Siena, Italy

To cite this Article Scarselli, M. , Vasco, A. , Bonci, A. , Rustici, M. and Niccolai, N.(1997) 'NMR STRUCTURAL INVESTIGATION OF THE BIOTIN-AVIDIN COMPLEX: A ^{13}C NMR PARAMAGNETIC RELAXATION STUDY', Spectroscopy Letters, 30: 6, 1201 — 1209
To link to this Article: DOI: 10.1080/00387019708006718
URL: http://dx.doi.org/10.1080/00387019708006718

SPECTROSCOPY LETTERS, 30(6), 1201-1209 (1997)

NMR STRUCTURAL INVESTIGATION OF THE BIOTIN-AVIDIN COMPLEX: A ^{13}C NMR PARAMAGNETIC RELAXATION STUDY

Keywords: Intermolecular interaction; paramagnetic relaxation; biotin; egg-white avidin; ^{13}C NMR.

M. Scarselli, A. Vasco, A. Bonci, M. Rustici and N. Niccolai

Contribution from the Dipartimento di Biologia Molecolare
Università di Siena, Pian dei Mantellini 44, 53100 Siena, Italy

ABSTRACT: The paramagnetic contributions to the spin-lattice relaxation rates of biotin ^{13}C nuclei, induced by the presence in the water/DMSO solution of the TEMPOL nitroxide, have been analysed in the interaction with avidin. The paramagnetic relaxation data, obtained at different temperatures, indicate that the average solvent/spin-label exposure of biotin carbons is consistent with the conformational features previously observed for the complex in the crystal. The analysis of the paramagnetic perturbation profiles, derived from ^{13}C spin lattice relaxation measurements, seems to be highly informative of the sterical aspects of interaction processes of large biopolymers with their ligands.

INTRODUCTION

The spin-labelling of the solvent molecules with free radicals such as the nitroxides has been proposed as a general method for obtaining information on surface accessibility of proton nuclei of peptides by observing the perturbation induced by the paramagnetic species on the proton spin-lattice relaxation rates[1,2]. More recently, the analysis of cross-peak intensity dependence of 2D protein spectra obtained at different spin-label concentration has been used to extend this approach for structural investigations of biopolymers[3-5].

The formation of intermolecular adducts between biological ligands and macromolecules may cause strong changes in the solvent exposure of molecular sites whose nuclear spin-lattice relaxation behaviour can be monitored in a spin-labelled solution where the binding of small molecules with DNA[6] and of peptides[7] with their receptors occurs. The extension of this methodology to protein systems with the analysis of the spin-label induced effects on ^{13}C spin-lattice paramagnetic relaxation is here presented. This 1D relaxation approach, or a multidimensional NMR analogue, should be used whenever the substrate complexity requires a resonance dispersion not offered by proton spectra.

The biological relevance of biotin has been recognised when its coenzyme function was discovered[8]. Furthermore, when avidin, a glycoprotein, which exhibits a very high affinity for this vitamin, was isolated, new methodological perspectives in biotechnology could be opened[9]. Biotin-depleted avidin is fairly thermostable, with a temperature of the folded/unfolded transition, t_m, of 85°C, in the pH range 7 to 9, almost irrespective of the buffer medium and/or of the ionic strength. Binding of biotin increases substantially the thermal stability of the complex (t_m = 132 °C) and the unfolding enthalpy[10]. Moreover, the avidin-biotin complex is stable in 9 M urea, in the pH range 2 to 13, and is not

denatured in 7 M guanidinium hydrochloride[11]. Thus, the avidin/biotin complex formation represents an interesting model system for testing new structural spectroscopical approaches for the delineation of the sterical aspects of intermolecular interactions in solution.

In the present report, the use of nitroxide paramagnetic probes has been explored for obtaining information on the structural features of the formation of the biotin-avidin complex as a model system for other substrate-enzyme interactions.

MATERIALS AND METHODS

D-Biotin, 2,2,6,6-tetramethyl-4-hydroxy-piperidine-N-oxyl (TEMPOL) and avidin were obtained from Sigma Chemical Company and used without further purification. Solutions were prepared by dissolving 15 mg of biotin, 20 mg of avidin in 0.5 ml DMSO-d_6/D_2O in 75:25 ratio, to have a 120 and 0,6 mM concentrations respectively for the two species. Paramagnetic solutions were prepared by adding TEMPOL to the biotin and biotin/avidin solutions to obtain a free radical concentration of 46 mM. Carbon partially relaxed spectra, obtained by using the Inversion Recovery pulse sequence, as well as all the other ^1H and ^{13}C NMR spectra were recorded with a Bruker AC-200 NMR spectrometer. Frequency assignments of proton and carbon resonances were reached from literature[12] and 2D heteronuclear correlation data respectively. Relaxation rates were calculated by computer fitting of relaxation curves and the calculated experimental errors were always lower than 5%.

RESULTS AND DISCUSSION

Due to the strong affinity ($K_d=10^{-15}$) of avidin with its substrate biotin[13], to favour fast exchange conditions all the NMR experiments were carried out at high temperature (50

°C), with organic solvent additions and at high biotin:avidin concentration ratio (50:1 for each of the four protein binding sites).

Fig. 1 shows the molecular model of D-biotin, built on the basis of the crystallographic data[14], and protonated carbon chemical shifts are given in Table I, according to the J connectivities found in the ^{13}C-^{1}H hetero-correlated 2D spectrum. The spin-lattice paramagnetic relaxation rates, R_p, defined as the difference between the relaxation rates measured in the presence and in the absence of TEMPOL, for the protonated carbons of biotin were calculated and reported in Fig. 2 as variances, v_i :

$$vi = 1 - \frac{Rpi}{\langle Rp \rangle} \qquad (1)$$

$$\langle Rp \rangle = \left(\sum_{i=1}^{n} Rpi \right) / n \qquad (2)$$

where R_{pi} is the difference between the relaxation rates respectively measured in the presence and in the absence of the paramagnetic probe and n is the number of the i nuclei whose spin lattice relaxation is followed.

As shown in Fig. 2, among the carbons of the rigid molecular moiety $C_{6'a}$ exhibits the highest v_i, in agreement with its surface accessibility, not much disturbed by the distant side chain. The interplay of differential segmental motions and surface accessibility yields, for the side chains carbon nuclei, effects whose absolute interpretation is not straightforward. However, the v_i profile is entirely determined by the biotin structure and the dynamics of the interaction between the diamagnetic and the paramagnetic solutes.

Upon addition of avidin in the ^{13}C NMR spectra of biotin the following features can be observed : i) no chemical shift

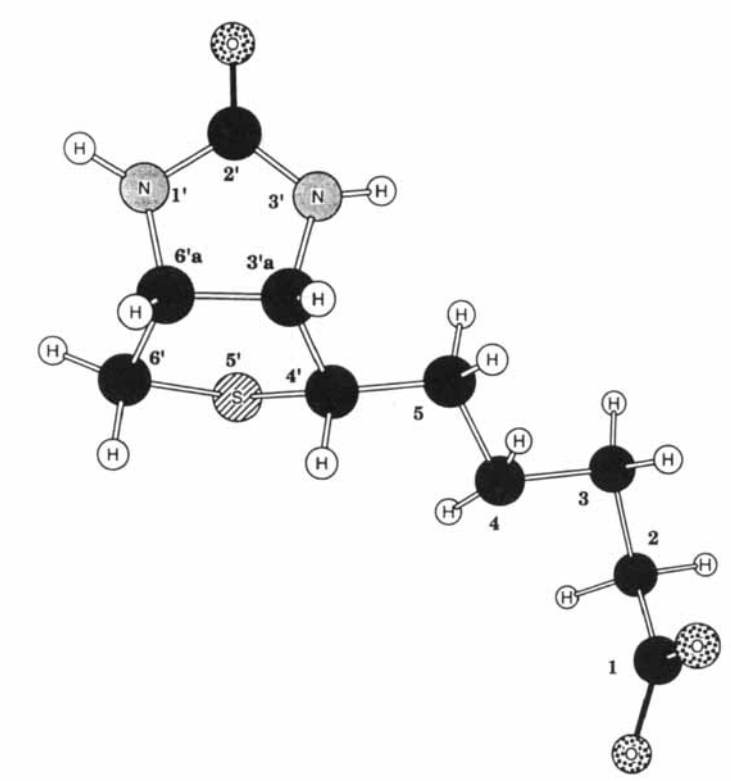

Fig. 1: The biotin structure, including the atom numbering which has been used in the discussion of protonated carbon spin lattice relaxation rates.

changes, ii) an increase of the diamagnetic relaxation rates induced by more hindered rotational motions, see Fig. 1, iii) a markedly changed paramagnetic relaxation profile, as shown in Fig. 2. The latter two aspects may be interpreted in terms of the occurrence of the interaction process of the substrate with the enzyme: in the fast exchange limit, the relaxation rates reflect, for each nucleus, averaged rotational motions and magnetic environments of free and bound biotin molecules.

Table I: Protonated carbon chemical shift, from the DMSO-d6 signal at 39.6 ppm, is reported, together with the respective diamagnetic spin lattice relaxation rates, R_i, measured at 50 °C and the thermal factor of the X ray study of ref. 14.

^{13}C n°	2	3	4	5	3'a	4'	6'	6'a
$\delta^{13}C$ (ppm)	34,77	25,40	28,87	29,04	62,43	56,22	40,20	60,57
R_i (s^{-1})	1.39	1.60	2.17	1.58	1.59	1.37	a	1.33
Ui	40.00	21.78	23.04	45.96	12.68	12.11	11.45	41.53

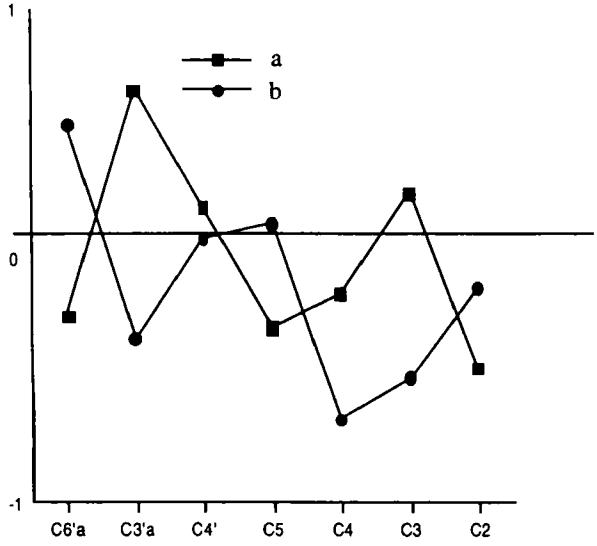

Fig. 2: The profile of the paramagnetic relaxation variances, v_i, as defined in the text and measured at 50 °C, for selected ^{13}C nuclei of biotin in the presence (a) and in the absence (b) of avidin.

From a comparison of the paramagnetic profiles shown in Fig. 2, two main differences are apparent: i) the paramagnetic perturbation on C2 is considerably reduced by the presence of avidin, ii) the paramagnetic effects exhibited by C3'a and C6'a show opposite trends in the presence and in the absence of avidin. The latter results suggest that C3'a and C6'a in the protein complex are located in positions with different surface accessibilities.

In order to obtain a molecular model for the biotin-avidin complex to confirm the consistency of the above described findings and interpretations, the available crystallographic data of the complex were considered.

In the crystal structure of the biotin-avidin complex, biotin turns out to be inserted differently in the two the avidin active sites, being the first a very deep hydrophobic pocket, while the second is in a relatively superficial moiety of the protein. In the first binding site a limited surface accessibility is observed especially for the biotin carbonylic group, the N atoms and C6'a of the ureidic ring, as the latter carbon is partially shielded by avidin W^{97} side chain. The other biotin C atoms, at similar extents, are solvent exposed, except the C2 atom of the valeric acid side chain.

The different accessibility of biotin atoms can be inferred by the thermal factors reported in the crystallographic study, see Table I. The dynamics of the molecular complex in the crystal is in agreement with the NMR derived information, as the thermal factor of C3'a is much larger than the one exhibited by C6'a.

These features support the validity of the solvent spin labelling NMR methods for analysing the conformational features of any interaction process which modulates the solvent accessibility of molecular moieties involved in the complex formation. The possibility of using also the ^{13}C nucleus for monitoring paramagnetic perturbation profiles extend the solvent spin labelling approach to large ligands and molecular complexes.

ACKNOWLEDGEMENTS

This work was supported by grants from MURST and the Italian CNR (Progetto Strategico Biologia Strutturale).

REFERENCES

1) N. Niccolai, G.Valensin, C. Rossi, and W.A. Gibbons, J.Am.Chem.Soc. 1982; 104; 1534.

2) N. Zhou, P. Mascagni, W.A. Gibbons, N. Niccolai, C. Rossi and H.Wyssbrod, J.Chem.Soc.Perkin II 1985; 581.

3) A.M. Petros, L. Mueller, and K.D. Kopple, Biochemistry 1990; 29; 10041.

4) N. Niccolai, G. Esposito, P. Mascagni, A. Motta, A. Bonci, M. Rustici, M. Scarselli, P. Neri, and H. Molinari, J. Chem. Soc. PERKIN II 1991; 1453.

5) G. Esposito, A.M. Lesk, H. Molinari, A. Motta, N. Niccolai, and A. Pastore, J. Mol. Biol. 1992; 224; 659.

6) N. Niccolai, R. Lampariello, L. Bovalini, P. Mascagni, and P. Martelli, Biophys. Chem. 1990; 380; 155.

7) E.T. Olejniczak, R.X Xu and S.W. Fesik, Journal of Biomolecular NMR 1992; 2(6); 655.

8) S.J. Wakil, E.B. Titchener, and D.M. Gibson, Biochim. Biophys. Acta 1975; 29; 85.

9) M. Wilcher, and E.A. Bayer,Meth. Enzymol. 1990; 184; 5.

10) J.W. Donovan, and K.D. Ross, Biochemistry 1973; 12; 512.

11) N.M. Green, Biochem. J. 1963; 89; 609.

12) Ikura and Ikichi Org. Magn. Reson. 1987; 20; 266.

13) N.M. Green, Adv. Protein Chem. 1975; 29; 85.

14) L. Pugliese, A. Coda, M. Malcovati, and M. Bolognesi, J. Mol. Biol. 1993; 231; 698.

Date Received: March 25, 1997
Date Accepted: April 30, 1997