Nuclear Magnetic Resonance Study of the Oxovanadium(IV)–(Glutathione)$_2$ Complex

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Abstract

^{13}C and ^{1}H NMR relaxation rates were measured for the glutathione–VO^{2+} 2:1 complex in aqueous solution. The kinetics of the dissociation of the peptide from the coordination sphere were delineated and the structure of the complex was determined. The two carboxyl groups were shown to be the main binding sites.

Introduction

Vanadium has been known for some years to be an essential trace element in both plants and animals [1], although its function is still unclear. The discovery that vanadate is a potent inhibitor of (Na, K)–ATPase [2] has aroused considerable interest in several vanadium-dependent physiological and biochemical effects, which yielded evidence that the predominant oxidation state of vanadium within tissues and cells is 4^{+}.

In fact in red cells VO_3^- is reduced almost quantitatively to VO^{2+}, which is tightly bound to hemoglobin [3]. During the study of vanadium biochemistry, a major role was discovered for the small endogenous peptide glutathione (GSH) which was found to be responsible for the reduction of VO^{2+} [3] and to act as a ligand for the VO^{2+} ions formed within adipocytes [3, 4].

This leads to consideration of model VO^{2+}–GSH complexes in aqueous solution, with the aim of evaluating the physico-chemical properties of the binding equilibrium. Since VO^{2+} has one unpaired electron with a non-degenerate ground state and a large energy separation between it and the excited states (due to the strong axial component of the crystal field created by the short V–O internuclear distance), paramagnetic resonance studies proved a suitable approach to the problem. In particular, shortening of nuclear spin relaxation rates by the paramagnetic VO^{2+} ions was expected to yield both kinetic and structural information.

Experimental

Stock solutions of reduced glutathione (Sigma) and $(VO)SO_4 \cdot 2H_2O$ (Merck) were prepared in D_2O and the pH was adjusted to the desired value with either DCl or NaOD. Preparation of samples, as well as pH adjustment, were carried out under a nitrogen atmosphere in order to prevent oxidation phenomena.

The NMR experiments were performed with a Varian XL-200 spectrometer operating in the FT mode at 200 MHz for ^{1}H NMR and 50.3 MHz for ^{13}C NMR. Longitudinal relaxation rates ($1/T_1$) were measured by using the inversion recovery pulse sequence. Transverse relaxation rates ($1/T_2$) were measured by using the Carr-Purcell-Meiboom-Gill pulse sequence. The T_1 and T_2 values were calculated from exponential regression analysis of the magnetization temporal dependence by using the computer of the spectrometer. The error of a single measurement was given as 95% confidence limits of the regression analysis.

Results and Discussion

Swift and Connick derived the relaxation time in a paramagnetic solution from a rigorous solution of the Bloch equation [5]. If the chemical exchange from the coordination sphere controls the relaxation process of the ligand nuclei, the observed relaxation rate T_{iobs}^{-1} (i = 1, 2) is related to the exchange rate of the ligand:

$$T_{iobs}^{-1} - T_{iF}^{-1} = T_{ip}^{-1} = f\tau_m^{-1} \quad (i = 1, 2) \qquad (1)$$

were T_{iF}^{-1} is the relaxation rate in the absence of metal ions, f is the fraction of ligand present in the

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coordinations sphere and τ_m^{-1} is the rate constant for dissociation of the ligand from the metal coordination sphere.

Alternatively, if the chemical exchange is rapid the relaxation process is controlled by the dipole-dipole or scalar coupling T_{iM} mechanism in the first or second coordination sphere of the VO^{2+} ion:

$$T_{ip}^{-1} = fT_{iM}^{-1} \quad (2)$$

The general Solomon–Bloembergen expression for T_{iM}^{-1} [6] provides a very useful way of obtaining structural information from the T_{iM}^{-1} values of different nuclei within the ligand molecule. The same kind of information can also be obtained when the chemical exchange is extremely slow, but in such cases the relaxation process is controlled by a T_{iM} mechanism involving the outer-sphere ligand molecules exchanging with a diffusion-controlled process.

Whether or not the chemical exchange controls the relaxation process can be tested by plotting $\ln T_{ip}^{-1}$ as a function of the reciprocal temperature. Equation 1 is applicable if the plot is linear with a negative slope, eqn. 2 is applicable if the plot is linear with the positive slope, being the outer sphere T_{iM} mechanism relevant in the low temperature region. Alternatively the T_{2p}^{-1}/T_{1p}^{-1} ratio can be considered: in fact in the exchange-controlled region $T_{2p}^{-1}/T_{1p}^{-1} = 1$ is expected. If this is not the case the T_{2p}^{-1}/T_{1p}^{-1} value can only be determined by eqn. 2, whereas the T_{2p}^{-1} value can still be determined by τ_M^{-1}.

When measuring the proton relaxation rates of GSH in aqueous solution the resonance from the α-proton of cysteine is masked by that of the residual water in the sample, such that the relative relaxation behaviour cannot be suitably recorded. The temperature dependence of the T_{2p}^{-1}s of the other proton resonance of GSH 100 mM in D_2O in the presence of $[VO^{+2}] = 10$ mM are reported in Fig. 1, where it is apparent that the fast exchange condition is never holding in the high-temperature field for any proton. However application of eqn. 1, together with the variation of τ_M with temperature given by [5].

$$\tau_M = \left(\frac{kT}{h}\right)^{-1} \exp(\Delta H^{\neq}/RT - \Delta S^{\neq}) \quad (3)$$

where h is the Planck constant, k the Boltzman constant and $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are the enthalpy and entropy of activation for the first-order reaction of exchange of GSH from the VO^{2+} ion, allows the evaluation of the kinetic constant for the exchange process ($k = \tau_m^{-1}$) as well as of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ for the different moieties within the peptide molecule, as reported in Table I. The exchange processes involve the whole GSH molecule and the rate of exchange is extremely slow if compared to the other VO^{2+} [7] complex, mainly due to a large entropy effect.

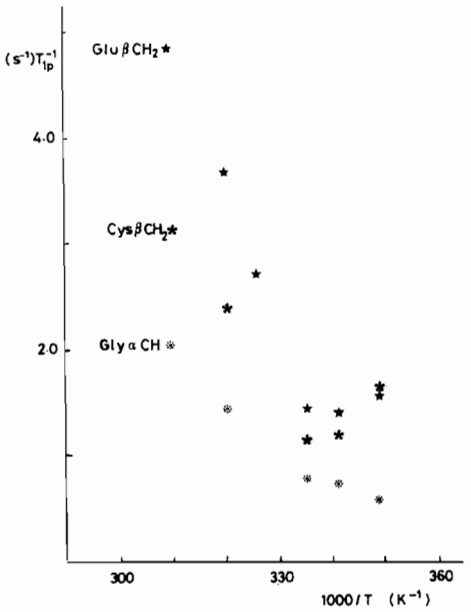

Fig. 1. Temperature dependences of T_{1p}^{-1} for selected protons of GSH 100 mM in D_2O. (VO^{2+}) = 10 mM. pH = 7.0.

TABLE I. Parameters Describing the Kinetics of Exchange of Glutathione from the Bound VO^{2+} Ion in a 2:1 Complex.

Moiety	τ_m (298 K) (s)	k' (298 K) (s^{-1})	$\Delta H^{\neq}$ (kcal/mol)	$\Delta S^{\neq}$ (u.c.)
GluβCH_2	0.13	7.5	5.4	−35.4
CysβCH_2	0.14	7.2	5.6	−34.9
Gly$_\alpha$-CH	0.26	3.8	6.8	−32.8

Elucidation of the structure of the complex could be attained by measuring the ^{13}C relaxation rates, as reported in Table II and in Fig. 2. Since the expressions for T_{1M} and T_{2M} contain an inverse six-power dependence on the metal–nucleus distance, the much shorter distances of the carbon nuclei to the VO^{2+} ion are expected to result into a T_{1M} (T_{2M}) determined relaxation process, which was shown to be the case. As a consequence the differential behaviour of the ^{13}C nuclei resulting into selective broadening of the ^{13}C resonance provides information about the proximity of the single carbons to the metal ion and, hence, on the binding sites within the GSH molecule. The extensive broadening of the Gly-COO^- and Glu-COO^- resonances, accompanied by sizeable broadening of those relative to the Gly-C_α, Glu-C_α and Glu-C_β, whilst the remaining resonances appear almost

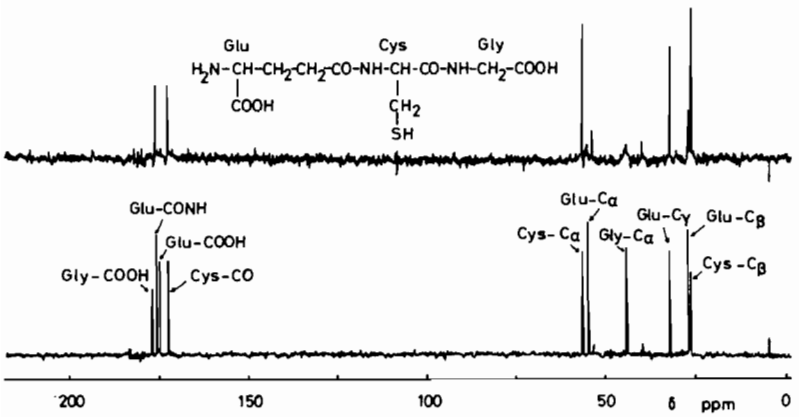

Fig. 2. ^{13}C NMR spectra of GSH 100 mM in D_2O (pH = 7.0. T = 298 K). The upper spectrum was recorded in the presence of (VO^{2+}) = 10 mM.

TABLE II. T_{1p}^{-1} and T_{2p}^{-1} Measured for ^{13}C Nuclei of GSH 100 mM in D_2O (VO^{2+}) = 10 mM, pH = 7.0, T = 298 K.

Resonance	T_{1p}^{-1}	T_{2p}^{-1}
Gly-COOH	u.b.[a]	u.b.[a]
Glu-CONH	4.42	10.71
Glu-COOH	u.b.[a]	u.b.[a]
Cys-CO	3.27	10.61
Cys-C_α	8.02	14.21
Glu-C_α	u.b.[a]	u.b.[a]
Gly-C_α	u.b.[a]	u.b.[a]
Glu-C_γ	18.24	56.12
Glu-C_β	17.16	49.75
Cys-C_β	10.26	32.40

[a]u.b. = undetectably broadened.

unaffected, can be taken as reliable proof of a structure involving four carboxyls in the equatorial position, as shown in Fig. 3. Such a conclusion is in good agreement with some ESR results [4] where the isotropic hyperfine coupling constant (Δ_{iso}) for the GSH–VO^{2+} 2:1 complex was measured at 102.6 G, similar to that measured for the VO–$(malonate)_2^{-2}$ complex [8].

Since for VO^{2+} complexes isotropic hyperfine coupling reflects the average environment around the VO^{2+} ion contributed by the four equatorial ligands [8, 9], it may be concluded that GSH and malonate are bound through the same donor groups.

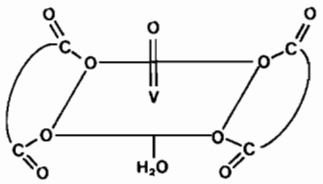

Fig. 3. Structure of the VO^{2+}. GSH 1:2 complex.

References

1 E. J. Underwood, 'Trace Elements in Human and Animal Nutrition', Academic Press, London, 1971, p. 416.
2 L. Josphson and L. C. Cantley jr., *Biochemistry, 16*, 4572 (1977).
3 I. G. Macara, K. Kustin and L. C. Cantley jr., *Biochim. Biophys. Acta, 629*, 95 (1980).
4 M. Degani, M. Gockin, S. J. D. Karlish and Y. Schechter, *Biochemistry, 20*, 5795 (1981).
5 T. J. Swift and R. E. Connick, *J. Chem. Phys., 37*, 307 (1962).
6 N. Niccolai, E. Tiezzi and G. Valensin, *Chem. Rev., 82*, 359 (1982).
7 H. Tomiyasu, S. Ito and S. Togami, *Bull. Chem. Soc. Jpn., 47*, 2843 (1974).
8 K. Wütrich, *Helv. Chim. Acta, 48*, 5306 (1965).
9 K. Wütrich, *Helv. Chim. Acta, 48*, 779 (1965).