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Electronic structure of vitamins D_2 and D_3

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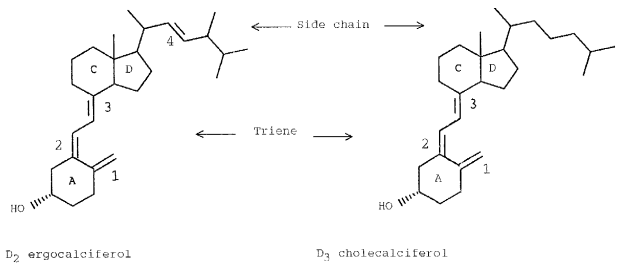
Abstract

The electronic structures of vitamins D_2 and D_3 were studied by He I photoelectron spectroscopy (UPS) and semi-empirical AM1 MO calculations. The analysis of electronic structure reveals two important facts. The vitamin D compounds have electronic structures which resemble steroid hormones and not other vitamins. Although biochemical behaviour and function of the title compounds has long been known to follow the pattern of steroid hormones, we demonstrate in this work that the electronic structures also closely resemble such hormones. The first ionization energies of vitamins D_2 and D_3 are unusually low (they are comparable to vitamin A derivatives) which raises the possibility that the title compounds may participate readily in various electron transfer processes. This aspect of vitamin D structure had so far been somewhat neglected in biochemical investigations. Also this work demonstrates that the electronic structure may play a significant role in the overall biochemical behaviour of some compounds.

Keywords: Vitamin D-2; Vitamin D-3; Nuclear receptor

1. Introduction

The medical and biochemical importance of vitamin D compounds have been well established for a long time [1–3]. The studies reported so far revealed many of their properties, especially metabolic pathways and conversions. D vitamins were classified on the basis of their synthesis and physiological activity, as steroid hormones. Their best known function is the regulation of Ca^{2+} and PO_4^{3-} levels in gut and kidneys (calcemic action). This, however, is not the only function of these versatile hormones and other roles are being currently investigated. Vitamin D_3 is metabolized into 1,25-$(OH)_2D_3$ which represents an active form of the vitamin and has a recognized nuclear receptor. The structural formulas of vitamin D_2 and D_3 reveal four distinct moieties within the molecule: ring A, C D rings, side chain and triene (numbers are used to label π bonds and corresponding orbitals π_1,π_2, π_3 and π_4).



The molecules are flexible and they can exist in many conformations in solution at room temperature.

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Ring A conformations are important for biological activity and mechanism of action of D vitamins [3], but, in spite of extensive investigations by theoretical [4] and experimental methods, some uncertainty remains about which conformers are predominant [5]. Furthermore, the most stable conformers are not necessarily the vehicles for biological action, which casts some doubt on the structure-function relationships [3]. Apart from very extensive conformational and structural studies on D vitamins, few studies on the formation of complexes between metals (Ca^{2+}, Cd^{2+}, Co^{2+}) and 1,25$(OH)_2D_3$ or other vitamin D ligands have been reported [6]. Electronic structure studies have not been reported at all which reflects the tacit assumption that their molecular skeleton (although flexible) is simply a carrier of functional groups which get attached to specific receptor sites. The aim of this work is to investigate this neglected aspect of their structure.

2. Materials and methods

The sample compounds were purchased from Fluka Chemie AG (> 99% purity declared and checked by HPLC). The spectra were recorded with a modified Perkin-Elmer PS 16 spectrometer using He I discharge. The spectra were measured with resolution < 30 meV at elevated sample inlet temperatures of 153 and 133°C for vitamins D_2 and D_3, respectively. Spectra were calibrated by adding small amounts of N_2O to the sample flow and using its 12.89 and 16.39 eV lines whose ionization energies are well known from their Rydberg series. No thermal decomposition was observed in the spectra as indicated by the absence of easily recognizable spectra of small molecules like CO, CO_2, H_2O, C_2H_4 etc. The AM1 and molecular mechanics calculations were performed with Spartan 4.0 program package [7]. The semi-empirical AM1 calculations used were of single-point type with preoptimization achieved by molecular mechanics (Sybyl force field). The choice of this level of calculations was prompted by the fact that π-ionizations can be readily identified in the low energy region; higher level calculations and Koopmans' approximation would not give better assignment, but would demand much longer computing times.

3. Results and discussion

He I photoelectron spectroscopy (UPS) [8] is the most direct available probe of molecular electronic structure, and because the measurements are performed in the gas phase no solvent effects will complicate data analysis. The measured spectra are shown in Fig. 1 and the assignments proposed on the basis of AM1 calculations and Koopmans' approximation are presented in Table 1. The π_1, π_2 and π_3 orbitals are localised on the respective double bonds of the triene system, while π_4 is the orbital localised on the side chain. Assignments given in Table 1 as well as empirical arguments based on comparison with spectra of trienes, suggest that all bands within ionization energies $E_i < 9$ eV, correspond to π-orbital ionizations which can be described as linear combinations of π_1, π_2 and π_3 basis orbitals.

The bands corresponding to ionization from lone pairs of hydroxyl group appear at $E_i > 9.5$ eV as do various σ orbitals. They were not assigned, due to the high density of states present and unreliability of Koopmans' approximation in the said energy range. The UPS spectra of vitamins D_2 and D_3 are different as can be clearly seen from Fig. 1. This reflects different electronic structures. The vitamins D_2 and D_3 are not identical molecules. The difference in the electronic structure, of course, stems mainly from the presence of additional double bond in D_2 and its concomitant interactions with other molecular orbitals. These interactions significantly influence total electron distributions in the two molecules; the difference of which is readily detectable by UPS. The interactions are a good example of the case when it is

Table 1
Ionization energies (E_i), MOs and assignments for vitamins D_2 and D_3

Compound	Band	E_i/eV	AM1 MO/eV	Assignment
D_2	$\tilde{X}$	7.55	8.72	$\pi_1 - \pi_2 + \pi_3$
	$\tilde{A}$	7.55	9.66	π_4
	$\tilde{B}$	8.70	9.98	$\pi_1 - (\pi_2 + \pi_3)$
	$\tilde{C}$	8.70	10.53	$\pi_1 + \pi_2 + \pi_3$
D_3	$\tilde{X}$	7.55	8.71	π_1-$\pi_2 + \pi_3$
	$\tilde{A}$	7.55	9.98	$\pi_1 - (\pi_2 + \pi_3)$
	$\tilde{B}$	8.54	10.53	$\pi_1 + \pi_2 + \pi_3$

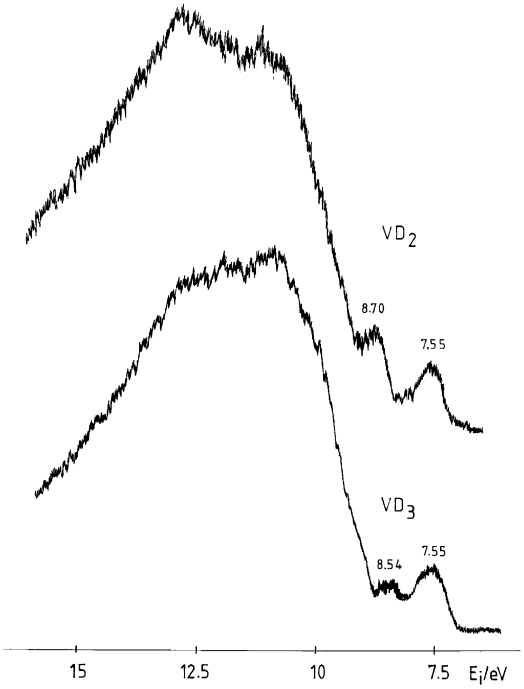

Fig. 1. He I photoelectron spectra of vitamins D_2 and D_3.

not satisfactory to simply reduce the structure of a large biomolecule to a collection of isolated functional groups linked by conformationally flexible joints.

The lowest energy bands in the spectra of vitamins D_2 and D_3 have maxima at 7.55 eV, which is an unusually low value. These E_i values are comparable to those recorded for vitamin A derivatives which are in the range 7.31–7.67 eV [9]. However, vitamin A derivatives contain long conjugated polyene chains, which are absent in vitamin D compounds.

The rationalization of such low E_i values cannot be sought in π-bond conjugation, because in the UPS of unconstrained [10] and sterically constrained trienes [11], the HOMO ionizations appear at 8.29–8.32 eV. Instead, the measured E_i values can be explained through interactions between π and high energy 'ribbon-like' σ orbitals. The existence of this type of interactions has been demonstrated in UPS of steroids [12]. An example of this 'relay type' orbital is shown in Fig. 2 for vitamin D_2.

Since such $\sigma-\pi$ interactions are common in steroids, our results give an independent confirmation (i.e., not deduced from biochemical function) that D vitamins really are steroid hormones. Vitamin D compounds can be considered as n-electron and π-electron donors, based on the presence of OH- and triene functional groups, respectively.

The low energy HOMO ionizations furthermore suggest that D vitamins, being n- and π-electron donors, can participate in intermolecular electron transfer processes; as vitamin A does. This information highlights a (so far) rather neglected aspect of vitamin D activity. The conformational analysis of vitamin D derivatives spanned 3 decades and the studies were reported in solid and liquid phases. Nonetheless, because of the molecules' flexible skeletons, uncertainties remain about the exact conformer

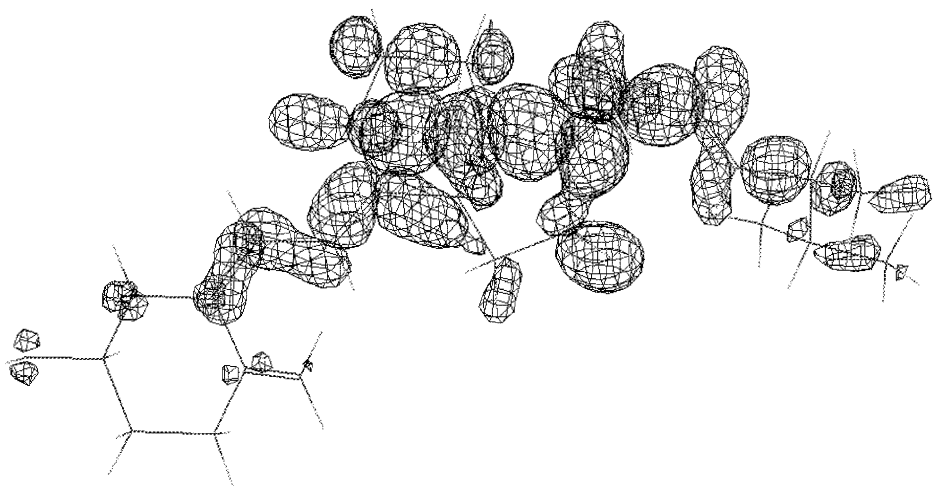

Fig. 2. An example of 'ribbon-like' high energy σ orbital in vitamin D_2.

population and relative biological activity of each. We have attempted to assess qualitatively the conformer range by using the approach outlined recently [13]. One can estimate the range of different conformers present by measuring relative widths of spectral bands. Vitamin D_3 spectrum exhibits slightly broader first band then vitamin D_2, with widths of 1.25 and 1.02 eV, respectively. The bandwidth difference is not due to instrumental factors, because the instrument resolution was checked before measurements of the sample spectra. A tentative suggestion is that vitamin D_3 has a more varied conformer population in the gas phase than D_2.

Low measured ionization energies suggest that the vitamins D_2 and D_3 may participate in various electron transfer processes where their electronic as well as molecular structure can determine biochemical function.

We shall give three examples (taken from studies of vitamin D properties by various techniques) as evidence that this may indeed be the case. Vitamins A, D_2 and D_3 were reported to be equally susceptible to rapid oxidation (at neutral pH) in the presence of lipoxygenase [14]. The extensive π-bond conjugation was not required for co-oxidation, but polar substituents were found to influence the rate. If one takes into consideration similar low values for ionization energies, the reported observations can be readily explained.

Vitamin D_3 was found to form very easily the charge-transfer complex with I_2 and this is the basis of a very sensitive spectrophotometric method for its determination [15]. Once again, our results are useful by suggesting that vitamin D_2 could be detectable by a similar method.

Finally, a recent study of the role of vitamin D_3 and its analogs [16] has shown that analogs without 1-OH group retain high affinity for calcium channel activation. This may imply that 1,25-$(OH)_2D_3$ metabolite does not bind to VDR protein receptor, but perhaps directly to the L-type calcium channel itself. The phenomenon may be the result of unusual electronic structure of vitamin D, its low E_i and perhaps partial electron transfer nature of the process.

In conclusion, we can say that the electronic structures of vitamins D_2 and D_3, as revealed by UPS, are similar but not identical. Their antirachitic activities are comparable [1] although the conformer populations may not be. Further study of the relation between biological activity and electronic structure would certainly be profitable as would the exploration of new possible (electron transfer?) modes of action of D vitamins. This work is an example of the need to sometimes include electronic structure analysis in the study of biochemical activity.

Acknowledgements

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References

[1] Georghiou, P.E. (1977) Chem. Soc. Rev. 6, 83–107.
[2] Okamura, W.H. and Wing R.M. (1980) in Vitamin D, Molecular Biology and Clinical Nutrition (Norman A.W., ed.), p. 59, Marcel Dekker, New York.
[3] Okamura, W.H., Midland, M.M., Hammond, M.W., Abd Rahman, N., Dormanen, M.C., Nemere I. and Norman, A.W. (1994) in Vitamin D, A Pluripotent Steroid Hormone, Structural Studies, Molecular Endocrinology and Clinical Applications (Norman, A.W., Bouillon, R. and Thomasset, M., eds.), W.de Gruyter, Berlin, p. 12.
[4] Hofer, O., Kahlig, H. and Reischl, W. (1993) Monatsh. Chem. 124, 185–198.
[5] Hofer, O., Kahlig, H. and Reischl, W. (1991) in Vitamin D, A Pluripotent Steroid Hormone: Structural Studies, Molecular Endocrinology and Clinical Applications (Norman, A.W., Bouillon, R. and Thomasset, M., eds.), W. de Gruyter, Berlin, p. 190.
[6] Do, X.T., Coquin, A-S., Megdad, S., Khan, M.A. and Bouet, G. (1994) in Vitamin D, A Pluripotent Steroid Hormone: Structural Studies, Molecular Endocrinology and Clinical Applications (Norman, A.W., Bouillon, R. and Thomasset, M., eds.), W. de Gruyter, Berlin, p. 37.
[7] SPARTAN, ver. 4.0, Wavefunction Inc. CA 92715, USA.
[8] Eland, J.H.D. (1983) Photoelectron Spectroscopy, 2nd edn., Butterworths, London.
[9] (a) Dougherty, D., Younathan, E.S., Voll, R., Abdulnur S. and McGlynn, S.P. (1978) J. Electron Spectrosc. Relat. Phenom. 13, 379-393. (b) Jericevic, Z., Klasinc, L., Kovac B. and Novak, I. (1980) Kem. Ind. (Zagreb) 29, 117–121.
[10] Bieri, G., Burger, F., Heilbronner E. and Maier, J.P. (1977) Helv. Chim. Acta 60, 2213–2233.
[11] Mollere, P.D., Houk, K.N., Bomse D.S. and Morton, T.H. (1976) J. Am. Chem. Soc. 98, 4732–4736.

[12] (a) Cvitas, T., Kovac, B., Pasa-Tolic, Lj., Ruscic, B., Klasinc, L., Knop, J.V., Bhacca, N.S. and McGlynn, S.P. (1989) Pure Appl. Chem. 61, 2139–2150. (b) Klasinc, L., Pasa-Tolic, Lj., Spiegl, H., Knop J.V. and McGlynn S.P. (1990) Comput. Chem. 14, 287–294.
[13] Novak, I. and Potts, A.W. (1996) J. Org. Chem. 61, 786–787.
[14] Gordon, M.H. and Barimalaa, I.S. (1989) Food Chem. 32, 31–37.
[15] Sanchez Perez, A., Galego Matilla, M.J. and Mendez Hernandez, J. (1993) Anal. Lett. 26, 721–731.
[16] Yukihiro, S., Posner, G.H. and Guggino, S.E. (1994) J. Biol. Chem. 269, 23889–23893.