Ph.D. Theses

Biospeciation of potential insulin-mimetic oxovanadium(IV) complexes

Ágnes Dörnyei

Supervisor:

Dr. Tamás Kiss

University of Szeged

Department of Inorganic and Analytical Chemistry

Szeged, 2008

Introduction and aim of the present work

The number of patients suffering *diabetes mellitus* (DM) is increasing year-by-year and it has risen to approximately 250 million worldwide in 2007. DM is defined as a disease that results in chronic hyperglycemia due to an absolute or relative lack of insulin and/or insulin resistance that in turn impairs glucose, protein and lipid metabolism, and finally entrains the characteristic secondary complications.

Trials for the use of vanadium in treating DM were first reported in 1899, which was 23 years before the discovery of insulin. However, pharmaceutical interest in vanadium as an insulin substitute started in the 1980s. The insulin-like effects of vanadium compounds were proved by *in vitro* and *in vivo* studies. The main advantage of these compounds to insulin is that they may be administered orally. Accordingly, the aim of the research in this field is to make vanadium compounds which can reach the target cells with high efficacy. According to our present views, these compounds should have low molecular mass, should be neutral and should have optimal lipophilicity in order to be mobile and cross cell membranes easily.

Most of vanadium's biologically important reactions occur in water-based environments such as blood plasma and intracellular fluids. Therefore the knowledge of the distribution and chemical speciation of the vanadium compounds in aqueous solution is of the utmost importance. It has to be taken into account that insulin-mimetic drugs will interact with numerous vanadofil exogenous or endogenous biomolecules during and after absorption. Via the redox and complexation reactions, the finely-tuned speciation of vanadium may lead to the efficiency of the metal to mimic the effects of insulin.

The primary aims of the present work have been (i) to study the aqueous equilibrium of $V^{IV}O$ – potential carrier ligand systems and (ii) to model the interactions and actual chemical form of the vanadium in cells.

Some carbohydrate and sal_2en-type Schiff base derivatives have been selected as potential carrier ligands. The group of aldaric acids has been chosen, because they or their derivatives are produced naturally in small amounts by mammals, including humans, and the arrangement of the two carboxylic and the alcoholic donors is suitable for metal ion binding. The diastereomeric D-glucaric and galactaric acids have been characterized. The other group, the sal_2en-type molecules present versatile steric, electronic and lipophilic properties. They may be easily prepared by the condensation of two compounds: (i) an aromatic o-hydroxyaldehyde and (ii) a diamine; the hydrophilic–lipophilic balance being easily fine-tuned by choosing the appropriate amine precursors and ring substituents of the aldehyde. The reduction of imine groups of the Schiff bases to amine groups gives beneficial properties to the sal_2en derivatives. The pyridoxal-containing pyr_2en and $rpyr_2$en, and the asymmetric sal_2dpa and sal_2orn bearing a pendant carboxylate group have been studied.

In order to assess the molecular form of vanadium insulin-mimetic complexes in cells, the interactions in the model systems of $V^{IV}O$-maltol and $V^{IV}O$-dipicolinic acid with various vanadofil cell components ATP and GSH also have been studied.

Accordingly, the solution speciation and the binding modes of the $V^{IV}O$ complexes of selected ligands have been investigated and discussed in the dissertation. pH-potentiometry has been used to determine the stoichiometry and stability constants of the complexes formed, and spectroscopic (UV-VIS, CD and EPR) measurements have been made to establish the most probable structures of the complexes present in aqueous solution.

Experimental methods

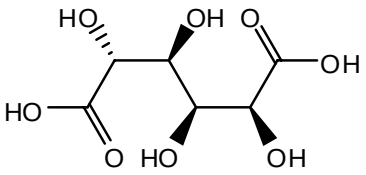
Solution equilibria, speciation and composition of species formed have been determined by pH-potentiometric titrations. Reactions causing some pH-effect can be investigated by this method. All measurements have been carried out in aqueous solution, at constant temperature (25.0 ± 0.1 ^{o}C) and ionic strength (I = 0.20 M, KCl), under inert atmosphere (Ar). Experimental data have been evaluated by SUPERQUAD and PSEQUAD computer programs.

The complex formation has been followed in all system by UV-Vis spectrophotometry. The evaluation of electronic absorption spectra gives some information about the number and the quality of the coordinating donor atoms and the geometry of the complex formed.

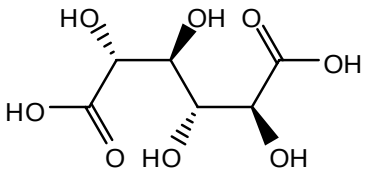
*C*ircular *D*ichroism spectroscopy (CD) is a very powerful technique to study optically active molecules. Circular dichroism or Cotton-effect is based on the differential absorption of left- and right-handed circularly polarized light in optically active medium. CD spectra in the visible light region are only produced when a metal ion is in a chiral environment. Optical activity in metal ion complexes has been attribute to configurational, conformational and the vicinal effects. This method has been used for characterizing the complexes formed in the $V^{IV}O$–sal_2-L-dpa system and for detecting some ternary complex formation with ATP or GSH.

*E*lectron *P*aramagnetic *R*esonance (EPR) spectroscopy is a technique to study paramagnetic ions or molecules. The oxovanadium(IV) ion has one unpaired electron (S = 1/2), while the isotope ^{51}V (its natural abundance is 99.76%) has a nuclear spin of 7/2; thus, the isotropic spectra obtained at room temperature consist of eight lines, while in frozen solutions $V^{IV}O$ complexes of tetragonal symmetry display eight parallel and eight perpendicular lines. The EPR parameters of the complexes have been obtained by simulation of the spectra with the aid of the computer program of A. Rockenbauer and L. Korecz. These data have been used to establish the most probable binding mode of the complexes formed.

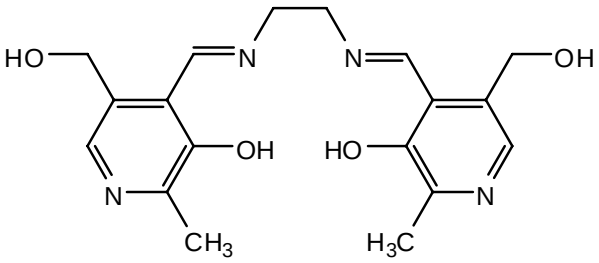
Structure of the ligands studied



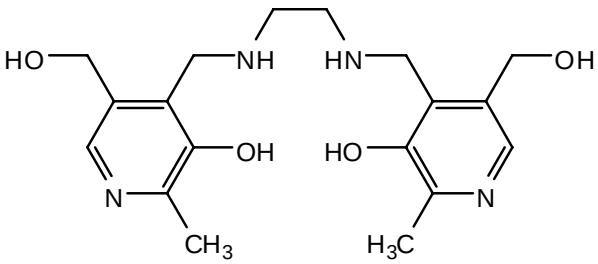
D-glucaric acid



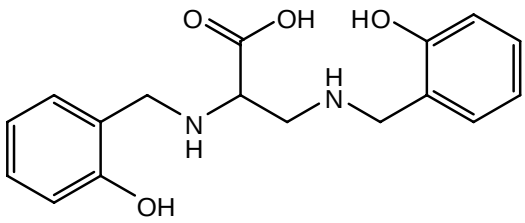
galactaric acid



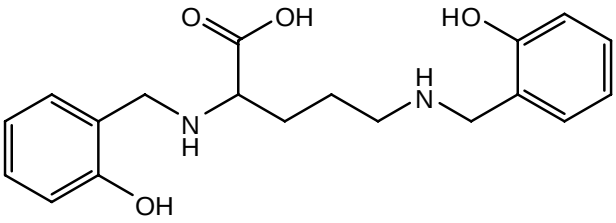
pyr₂en: N,N'-ethylene-bis(pyridoximine)



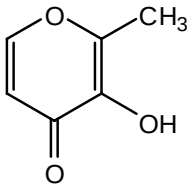
rpyr₂en: N,N'-ethylene-bis(pyridoxamine)



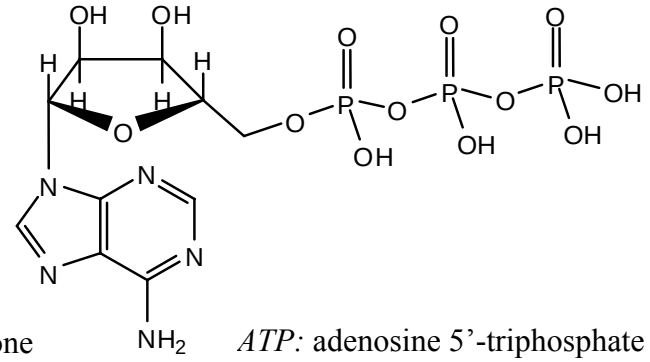
sal₂dpa: N,N'-bis(2-hydroxybenzyl)-2,3-diamino-propionic acid



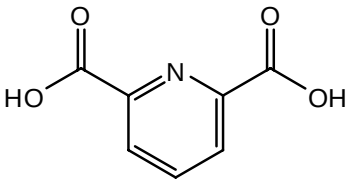
sal₂orn: N,N'-bis(2-hydroxybenzyl)-2,5-diamino-pentanoic acid



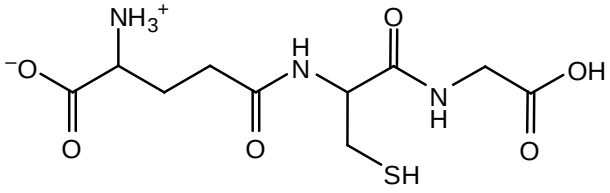
maltol: 3-hydroxy-2-methyl-4-pyrone



ATP: adenosine 5'-triphosphate



dipicolinic acid:
pyridine-2,6-dicarboxylic acid



GSH:
reduced glutathione, γ-Glu-Cys-Gly

New scientific results

1. *Oxovanadium(IV) complexes of selected aldaric acids (D-glucaric and galactaric acids)*

   1.1. Both of the aldaric acids studied proved to be fairly strong vanadium binders in the weakly acidic and neutral pH range. At basic pH, the ligand molecules are partly displaced by OH^-. Accordingly of the stability of their complexes, they might promote the absorption of the vanadium as carrier ligands.

   1.2. At low pH, complex formation starts at the terminal COO^- functions and probably the adjacent alcoholic-OH groups strengthen the interactions between the metal ion and the ligand. Then the alcoholic-OH group in the coordination sphere deprotonates. This metal-induced process becomes complete with appearance of the dinuclear or dimer complexes. The dimerization is endothermic and entropy governed. An interesting *cis–trans* isomeric equilibrium characteristic for the α-hydroxycarboxylic acids has been assumed and analyzed by EPR and molecular modeling.

   1.3. These chiral ligands do not exhibit stereoselectivity in protonation reactions; meanwhile for the formation of dinuclear complexes the opposite has been established. According to the pH-potentiometric and EPR investigations the D-glucaric acid is a somewhat more efficient $V^{IV}O$ binder than the galactaric acid.

2. *Oxovanadium(IV) complexes of pyr$_2$en: N,N'-ethylene-bis(pyridoximine) and rpyr$_2$en: N,N'-ethylene-bis(pyridoxamine)*

   2.1. Both pyr$_2$en and rpyr$_2$en have been found to form basically similar 1:1 complexes ($[VOLH_n]^{n+}$; n = 2, 1, 0), the tetradentate coordination of the ligands through two phenolate-O and two amine/imine-N being the predominant binding mode. The pyridinium-N atoms of the pyridoxal rings do not take part in the coordination but are involved in acid-base reactions.

   2.2. The neutral [VOL] complex is hardly soluble in water, precipitations of these compounds occur during the pH-potentiometric titrations if their concentrations are in the mM range. The neutral complex of the pyr$_2$en is less soluble.

   2.3. The solution speciation revealed that the rpyr$_2$en forms more stable complexes with $V^{IV}O$ than the pyr$_2$en itself. This difference in the stability may probably be explained by the much higher flexibility of the reduced Schiff base ligand, lacking the –C=N– double bonds, thus resulting in significantly less strain and/or better ligand-metal orbital overlap in the complexes formed. At low pH, the pyr$_2$en forms $[VOLH_4]^{4+}$ complex first, only the half of the molecule coordinates through a phenolate-O and an imine-N. The pyr$_2$en ligand itself may also hydrolyze as other Schiff bases.

2.4. EPR studies indicate the presence of various isomeric species in the solution of the $V^{IV}O$ – $rpyr_2en$ system. An interesting *cis–trans* isomeric equilibrium has been assumed and analyzed by EPR and molecular modeling for the $[VOLH_n]^{n+}$ complexes.

2.5. Upon comparison with the $V^{IV}O$ –, Zn^{II} –, Ni^{II} – and Cu^{II} – $rpyr_2en$ systems, the proton displacement constants of the $[MLH_n]^{n+}$ complexes give the stability order $Cu^{II} > V^{IV}O > Ni^{II} > Zn^{II}$.

3. *Oxovanadium(IV) complexes of sal_2dpa: N,N'-bis(2-hydroxybenzyl)-2,3-diamino-propionic acid and sal_2orn: N,N'-bis(2-hydroxybenzyl)-2,5-diamino-pentanoic acid*

3.1. Both ligands form stable and differently protonated basically 1:1 complexes with $V^{IV}O$ ion in aqueous solution. The sal_2dpa and the sal_2orn proved to be efficient vanadium binder between pH 5–9 and 7–10, respectively, the $[VOL]^-$ is the only species in these pH ranges.

3.2. The sal_2dpa proved to be more efficient $V^{IV}O$ chelator than the sal_2orn, the arrangement of its donor atoms is more suitable for metal ion binding. We have highlighted the differences in the binding abilities of this pentatdentate sal_2dpa and other water soluble but tetradentate sal_2en derivative ($R(SO_3\text{-}sal)_2en$), coordination of the extra carboxylate group increases the stability of the complexes.

3.3. At low pH only the half of the molecules is coordinated tridentately, then two other donor groups deprotonate cooperatively and coordinate to the metal centre. EPR studies indicate the presence of various isomeric species in the solution of the $V^{IV}O$ – sal_2dpa system.

4. *Interactions of insulin-mimetic oxovanadium(IV) complexes with the cell constituents ATP and GSH*

4.1. Although both cell constituents form some ternary complexes in the model systems studied, the results suggest that GSH mostly takes part in redox reactions of vanadium and ATP, as a strong $V^{IV}O$ binder, chelates the metal ion, forming binary and/or ternary complexes.

4.2. ATP coordinates to $V^{IV}O$ centre through the phosphate moiety in the VOAB ternary complexes formed in the acidic and neutral pH range, and through the ribose residue in the $VOABH_{-2}$ complexes formed in basic solutions. At the physiological pH the $V^{IV}O$ ion rather forms biscomplex with maltol than ternary complexes with maltol and ATP. According to the speciation studies, in the solution of the $V^{IV}O$–dipicolinic acid – ATP system ternary complexes dominate between pH 4–9. The dipicolinic acid and the ATP bind more efficiently the vanadium together than separately.

4.3. Ternary complex formation with GSH has been detected only by EPR measurements at a relatively high excess of GSH. The lower excess of GSH applied in the pH-potentiometric titrations has not been enough to prevent the partial hydrolysis of the metal ion. In the ternary complexes of GSH, it most probably coordinates at the Gly end through (COO^-, amide-O or H_2O) donors and the participation of the S donor in the metal binding is possible above pH 7.

4.4. The results of this work strongly suggest that ATP binds relevant $V^{IV}O$ species under cellular conditions and thus might somehow be involved in the insulin-mimetic action of $V^{IV}O$ compounds. The time course of the parallel redox and complexation reaction needs further investigation.

Publications related to the subject of the dissertation

1. **Á. Dörnyei**, E. Garribba, T. Jakusch, P. Forgó, G. Micera, T. Kiss:
   Vanadium(IV,V) complexes of D-saccharic and mucic acids in aqueous solution
   Dalton Trans. **2004**, *12*, 1882–1891. IF: 2.926

2. I. Correia, J. Costa Pessoa, M. T. Duarte, R. T. Henriques, M. F. M. Piedade, L. F. Veiros, T. Jakusch, T. Kiss, **Á. Dörnyei**, M. M. C. A. Castro, C. F. G. C. Geraldes, F. Avecilla:
   N,N'-ethylenebis(pyridoxylideneiminato) and N,N'-ethylenebis(pyridoxylaminato): synthesis, characterization, potentiometric, spectroscopic and DFT studies of their vanadium(IV) and vanadium(V) complexes
   Chem.-A Eur. J. **2004**, *10*, 2301–2317. IF: 4.517

3. J. Costa Pessoa, S. Marcão, I. Correia, G. Gonçalves, **A. Dörnyei**, T. Kiss, T. Jakusch, I. Tomaz, M. M. C. A. Castro, C. F. G. C. Geraldes, F. Avecilla:
   Vanadium (IV and V) complexes of reduced Schiff bases derived from the reaction of aromatic o-hydroxyaldehydes and diamines containing carboxylic groups
   Eur. J. Inorg. Chem. **2006**, *18*, 3595–3606. IF: 2.704

4. **Á. Dörnyei**, S. Marcão, J. Costa Pessoa, T. Jakusch, T. Kiss:
   Interactions of insulin-mimetic vanadium complexes with the cell constituents ATP and glutathione
   Eur. J. Inorg. Chem. **2006**, *18*, 3614–3621. IF: 2.704

ΣIF: 12.851

Other publications

1. T. Jakusch, **Á. Dörnyei**, I. Correia, L. M. Rodrigues, G. K. Tóth, T. Kiss, J. Costa Pessoa, S. Marcão:
   Interaction of $V^{IV}O$, $V^{V}O_2$ and Cu^{II} with a Peptide Analogue SalGly-L-Ala
   Eur. J. Inorg. Chem. **2003**, *11*, 2113–2122. IF: 2.482

2. **Á. Dörnyei**, M. Kilyén, T. Kiss, B. Gyurcsik, I. Laczkó, A. Pécsváradi, M. L. Simon, M. Kotormán:
   The effects of Al(III) speciation on activity of trypsin
   J. Inorg. Biochem. **2003**, *97*, 118–123. IF: 2.343

3. **Á. Dörnyei**, I. G. Csizmadia:
   An exploratory study of the conformational intricacy of selected fluoro-substituted carboxylic acids
   J. Mol. Struc.-Theochem **2003**, *666–667*, 135–141. IF: 1.027

4. I. Correia, **A. Dornyei**, F. Avecilla, T. Kiss, J. Costa Pessoa:
   X-ray crystal structure and characterization in aqueous solution of {N,N'-ethylenebis(pyridoxylaminato)}zinc(II)
   Eur. J. Inorg. Chem. **2006**, *3*, 656–662. IF: 2.704

5. I. Correia, **A. Dornyei**, T. Jakusch, F. Avecilla, T. Kiss, J. Costa Pessoa:
   Water-soluble sal_2en- and reduced sal_2en-type ligands: study of their Cu^{II} and Ni^{II} complexes in the solid state and in solution
   Eur. J. Inorg. Chem. **2006**, *14*, 2819–2830. IF: 2.704

6. T. Kiss, T. Jakusch, D. Hollender, **Á. Dörnyei:**
   Biospeciation of insulin-mimetic VO(IV) complexes
   ACS Symposium Series 974, Vanadium: The Versatile Metal, Editors: K. Kustin, J. Costa Pessoa, D. C. Crans, **2007**, 323–339.

7. T. Kiss, T. Jakusch, D. Hollender, **Á. Dörnyei**, É. A. Enyedy, J. Costa Pessoa, H. Sakurai, A Sanz-Medel:
   Biospeciation of insulin-mimetic VO(IV) complexes
   Coord. Chem. Rev. **2008**, xx, xxx–xxx. (accepted for publication) IF: 8.815

ΣIF: 20.075

Oral (o) and poster (p) presentations

1. **Á. Dörnyei**, M. Kilyén, T. Kiss, B. Gyurcsik, I. Laczkó, A. Pécsváradi, M. L. Simon, M. Kotormán:
   The effects of Al(III) speciation on activity of trypsin (p)
   Fifth Keele Meeting on Aluminium; Aluminium in Life: From Acid Rain to Alzheimer's Disease, 23–25 February, 2003, Keele, UK

2. **Á. Dörnyei**, M. Kilyén, T. Kiss, B. Gyurcsik, I. Laczkó, A. Pécsváradi, M. L. Simon, M. Kotormán:
   The effects of Al(III) speciation on activity of trypsin – 2. Similarity searches (o)
   The Vth International Symposium – Young People and Multidisciplinary Research, 6–7 November, 2003, Timisoara, Romania

3. **Dörnyei Á.**, E. Garribba, Jakusch T., Forgó P., Kiss T.:
   A D-cukorsav és a nyálkasav vanádiumkötő sajátságai (o)
   XXXVIII. Komplexkémiai Kollokvium, 21–23 May, 2003, Gyula

4. **Á. Dörnyei**, G. Paragi, I.G. Csizmadia:
   An exploratory study of the conformational intricacy of selected fluoro-substituted carboxylic acids (p)
   "The role of chemistry in the evolution of molecular medicine" A tribute to Professor Albert Szent-Györgyi, 27–29 June, 2003, Szeged

5. **Dörnyei Á.**, E. Garribba, Jakusch T., Forgó P., G. Micera és Kiss T.:
   Aldársavak oxovanádium(IV)- és vanádium(V)-komplexei vizes oldatban (o)
   XXVI. Kémiai Előadói Napok, 27–29 October, 2003, Szeged

6. **Dörnyei Á.**, Kilyén M., Gyurcsik B., Laczkó I., L. Simon M., Kotormán M., Pécsváradi A., Kiss T.:
   Al(III)-komplexek hatása a szerin-proteáz tripszinre (o)
   XXXIX. Komplexkémiai Kollokvium, 26–28 May, 2004, Gárdony

7. **Á. Dörnyei**, T. Jakusch, P. Forgó, E. Garribba, G. Micera, T. Kiss:
   VO(IV)- and VO_2(V)-binding abilities of saccharic and mucic acids (o)
   3rd Working-group meeting (Insulin-mimetic vanadium compounds, COST D21/009/01), September 6–7, 2004, Szeged

8. **Á. Dörnyei,** S. Marcão, J. Costa Pessoa, T. Kiss:
   Complexation in the ternary systems of VO(IV)-dipicolinate and VO(IV)-maltolate with ATP and glutathione: a model for cell processes (o)
   4th Working–group meeting (Insulin–Mimetic Vanadium Compounds, COST D21/009/01), October 1–2, 2005, Thessaloniki, Greece

9. T. Kiss, **Á. Dörnyei**, S. Bouhsina, S. Marcão, J. Costa Pessoa:
   Interactions of insulin mimetic vanadium complexes with cell constituents: ATP and glutathione (p)
   First European Conference on Chemistry for Life Sciences, October 4–8, 2005, Rimini, Italy

10. **Dörnyei Á.**, S. Marcão, J. Costa Pessoa, Kiss T.:
    Inzulinutánzó oxovanádium(IV)komplexek kölcsönhatása sejtalkotó bioligandumokkal (o)
    XLI. Komplexkémiai Kollokvium, 31 May – 2 June, 2006, Mátrafüred

11. T. Kiss, T. Jakusch, D. Hollender, **Á. Dörnyei:**
    Biospeciation of insulin-mimetic VO(IV) complexes (o)
    The Fifth International Symposium on the Chemistry and Biological Chemistry of Vanadium, September 10–14, 2006, San Francisco, California, USA

12. **Á. Dörnyei**, T. Kiss, S. Marcão, J. Costa Pessoa:
    Interactions of insulin mimetic vanadium complexes with cell constituents: ATP and glutathione (p)
    The Fifth International Symposium on the Chemistry and Biological Chemistry of Vanadium, September 10–14, 2006, San Francisco, California, USA

13. J. Costa Pessoa, S. Marcão, I. Correia, G. Gonçalves, **A. Dörnyei**, T. Kiss, T. Jakusch, I. Tomaz, M.M.C.A. Castro, C.F.G.C. Geraldes, F. Avecilla:
Vanadium (IV and V) complexes of reduced Schiff bases derived from the reaction of aromatic o-hydroxyaldehydes and diamines containing carboxylic groups (p)
The Fifth International Symposium on the Chemistry and Biological Chemistry of Vanadium, September 10–14, 2006, San Francisco, California, USA

14. **Á. Dörnyei**, S. Marcão, J. Costa Pessoa, T. Jakusch, T. Kiss:
Interactions of insulin-mimetic vanadium complexes with the cell constituents ATP and glutathione (p)
2nd European Conference on Chemistry for Life Sciences,
September 4-8, 2007, Wroclaw, Poland