Theses of Ph. D. Dissertation

Biomimetic Modelling
Of
Biological Oxidations

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INTRODUCTION

My Ph. D. dissertation summarizes my scientific research activity started during my university studies at the Department of Organic Chemistry and first continued at CHINOIN Ltd. and later at Gedeon Richter Ltd.

As the first step of my work with the help of synthetically prepared metalloporphyrins (Figure 1.) I modelled the processes still unapplied in the research of insecticides and catalysed by cytochrome P450 playing an important role in the development of resistance against insecticides. I investigated the carbamate family of insecticides for the adjustment of the modelling system.

![Applied synthetic metalloporphyrins](image)

**Figure 1.** Applied synthetic metalloporphyrins

By the help of my investigations I searched for the answers to the following questions: (i) is the chemical model able to reproduce the oxidative metabolism of carbamate insecticides catalysed by cytochrome P450; (ii) does the model make possible to determine the metabolically sensitive groups of the
molecule; (iii) is a modelling system able to reproduce the metabolite profile provided in \textit{in vivo} experiments?

As the following step, using the chemical model system I studied the insect-selective cytochrome P450 inhibitors developed by CHINOIN Insecticide Sector. My aim was to identify the interaction forming between the inhibitors and cytochrome P450, and the mechanism of the possible biomimetic conversions.

Later, by the help of arginin, \(N\)-hydroxy-arginin and their structural analogues I investigated the NO synthesis catalysed by NOS that is also a hem protein. The goals of my investigations were the more precise understanding of the mechanism of NO synthesis and the development of a modelling system that is able to predict the NO donor property of an active substance.

In the second part of my work I investigated the interaction between Zn-hyaluronan that is Gedeon Richter Ltd.’s active substance, as well as the associates formed by hyaluronic acid (Figure 2.) with other metallic cations (\(\text{Na}^+\), \(\text{Co}^{2+}\), \(\text{Cu}^{2+}\), \(\text{Mn}^{2+}\)) and the metabolites containing the most important reactive oxygen from pathological point of view (ROM: hydroxyl (\(\text{OH}\)), superoxide anion (\(\text{O}_2^-\)), alkylperoxide (\(\text{RO}_2^-\)), peroxynitrite (\(\text{ONOO}^-\))).

![Figure 2. The disaccharide unit of hyaluronan](image)
My investigations were directed to getting answers to the following problems: (i) demonstration of the independent antioxidative effect of hyaluronan (HA); (ii) the exploration of the correlation between the degradating effect of ROMs exerted on HA and the independent antioxidative effect of HA; (iii) the demonstration of the metallic cation’s role in HA-associate’s antioxidative effect and of its potential protective effect in degradation processes against ROMs.

Beyond identification of the chosen biological oxidative processes and explanation of their mechanisms, the aim of my investigations was the installation of modelling systems that can be made applicable for quick and effective testing of new active substances because of their easily variable parameters and accessibility.

**SCIENTIFIC THESES**

1. During the investigations of carbamate insecticides (carbofuran, carbaryl, primicarb (Figure 3.)) the FeTPPF_{20} / m-chloro-perbenzoic acid (m-CPBA) and FeTPPF_{20}/H_{2}O_{2} biomimetic model systems developed by our research group, were proved to be suitable for modelling of processes catalysed by cytochrome P450.

![Figure 3. Tested carbamate insecticides](image)

Figure 3. Tested carbamate insecticides
We were able to point at the metabolically sensitive groups of all three carbamate insecticides and the received metabolite profiles proved good coincidence with the data originated from \textit{in vivo} experiments on house flies. In our tests we succeeded to clearly identify certain products of the oxidative mechanisms of carbaryl and primicarb.

2. The developed modelling system proved to be usable also for the testing of Verbutine and Perbutine (Figure 4.), the two insect-selective CP450 inhibitors that was prepared in CHINOIN.

![Figure 4. The structure of Verbutine and Perbutine](image)

Using the biomimetic system it was managed to expose those points of to the inhibitors that are sensitive to processes catalysed by cytochrome P450. The results originated from the two methods showed proper coincidence. Furthermore, it was also pointed out that the inhibitors underwent also alternative conversions in the chemical model reactions that can be explained only by processes catalysed by peroxidases (LiP, MnP). Based on these findings we were able to show the potential environmental advantages of application of the two inhibitors.

3. The FeTPPF$_{20}$/H$_2$O$_2$ biomimetic system was successfully used also for modelling the NO synthesis catalysed by NOS. Our results well conceded with the process
of iNOS that takes place in the presence of H$_2$O$_2$. Based on the results it can be stated that our modelling system would be applicable for the biomimetic oxidation of guanidine derivatives, as well as for the identification of potential NO-donors.

4. It was demonstrated in our experiments investigating the antioxidative effect of HA associates that HA (Na-HA) proves independent antioxidative effect only against ‘OH and ONOO’. This property presumably can be related to the degradation taking place in its structure („self-sacrificing antioxidative property”). The received curve prescribing the antioxidative effect, well correlated with the curve demonstrating the extent of HA degradation induced by ROMs separately.

The reasons of the antioxidative effect of HA associates, and of their effect against ROM-induced degradation that is superior to the effect of Na-HA, are seen at two points: (i) the effect can be originated from the redox property of metallic ions with varying oxidative states(Co$^{2+}$, Cu$^{2+}$ and Mn$^{2+}$). The existence of this effect can be seen in case of the effect of Cu-, Mn-HA and Cu$^{2+}$, Mn$^{2+}$ against O$_2^·$ and of Co-, Mn-HA and Co$^{2+}$, Mn$^{2+}$ against AAPH, as well as of Co-, Cu-HA and Co$^{2+}$, Cu$^{2+}$ against ONOO$. It is visible that in these cases not only the enhancement of the antioxidative effect can be seen, but also the protective effect of Co$^{2+}$, Cu$^{2+}$ and Mn$^{2+}$ on HA against ROM-induced degradation; (ii) the other potential reason can be related to the complex binding forming between HA and metallic ions. It is supported by several studies demonstrating the very close interaction forming between metallic ions and HA. Taking into consideration the independent pharmacological effect of the individual metallic ions, HA associates may have a significant role in the
treatment of diseases accompanied by the occurrence of increased ROMs (rheumatoid arthritis, chronic wounds etc.) where the restoration of HA’s rheological properties can be important as well as the elimination of developing ROMs.

REFERENCES

Publications referred to in the dissertation


Publications not referred to in the dissertation


Presentations, posters referred to the dissertation


7. Gy. T. Balogh, J. Illés, E. Forrai, Zs. Székely: Counter-ion effect on oxidative damage and free radical scavenger capacity of hyaluronic acid. Hungarian-German-Italian-Polish Joint Meeting on Medicinal Chemistry, Budapest September 2-6, 2001. (poster)