Final report for the project ”Green ” Antioxidants (ÅForsk 16-364)

Summary of results

In the project we have prepared a variety of novel organoselenium (nitro-, azo- and amino substituted Ebselen derivatives) and organotellurium (tellurobistocopherols and 2-aryltellurophenols substituted in the aryltelluro or phenolic part of the molecule) antioxidants. The novel compounds were found to act as excellent quenchers of peroxyl radicals. Furthermore, in the presence of suitable co-antioxidants, they could be continuously regenerated and act in a catalytic fashion. Some of them (Ebselens) were also found to efficiently catalyze the reduction of hydrogen peroxide in the presence of a thiol co-antioxidant. Mechanisms for the reduction of peroxyl radicals and hydrogen peroxide were proposed in the light of calculated O-H bond dissociation energies, deuterium labelling experiments, studies of the consumption of co-antioxidant and ^{77}Se NMR experiments.
The global market for antioxidants is enormous. Unfortunately, the large majority of these compounds act only on a stoichiometric basis. Thus, each molecule of a phenol or aromatic amine can only quench two peroxyl radicals before it is all consumed and gone. It would be much better if one could improve the chain-breaking capacity of the antioxidant and then regenerate it with some cheap, non-toxic co-antioxidant and make it perform in a catalytic fashion. Then one could cut down on the use of eco-unfriendly aromatic amines and phenols that continuously leak out in our environment today. Such catalytic antioxidants - properly described as “sustainable” and “green” - have been prepared and studied in the present project. We thank Å-Forsk for their generous support.

Research group and collaborations

Two PhD students (Jia-fei Poon and Jia-jie Yan) have been involved in the project. Jia-fei Poon, defended his thesis in October 2016. Jia-jie Yan defended his thesis in October 2017. A postdoc, Dr Vijay Pal Singh, has also been involved in the design, synthesis and evaluation of novel antioxidants. Dr Marjam Ott and her postdoc Dr Xi Lu, Department of Engineering Sciences, Kemi – Ångström, Uppsala University, have been involved in testing some of the antioxidants prepared for their toxicity and ability to reduce the production of reactive oxygen species in mononuclear cells. Paul Gates, University of Bristol, has been involved in the characterization of new antioxidants by high resolution mass spectrometry (HRMS). Prof. Ray J. Butcher, Howard University, USA, has kindly determined the structure of two organoselenium compounds. Dr. Henrik Ottosson and Kjell Jorner, Department of Chemistry – Ångström, has assisted us in the elucidation of antioxidant mechanisms by calculating O-H bond dissociation energies and transition state energies. Clara Schäfer and Carsten Donau from Germany have done project and examination works in the group As a supervisor, I have spent ca. 50 % of my time in the present project.

Scientific output

Described in Publication 1 is the syntheses of three tellurobistocopherols and an evaluation of their catalytic chain-breaking activities in a two-phase (water/chlorobenzene) lipid peroxidation system reminiscent of a biological membrane. The most active compound efficiently inhibited peroxidation for 742 min in the presence of aqueous-phase *N*-acetylcysteine as a co-antioxidant. This is the longest inhibition time we have ever recorded in this test system. For the first time, we also monitored thiol consumption in the aqueous phase while peroxidation was ongoing in the lipid phase. We found that the inhibition-time was inversely related to the rate of thiol consumption and that the availability of aqueous-phase thiol is the limiting factor for the duration of the antioxidant

protection in the chlorobenzene layer.
As outlined in our application, we also have an interest in organoselenium antioxidants. In man the glutathione peroxidases (GPx) are one of the most important hydroperoxide-decomposing enzymes. The reports on the existence of Gpx triggered a search for small-molecule compounds that could mimic the action of the large enzyme. The benzisoselenazolone ebselen was the first compound of this kind. Since the mid 1980s, the suitability of ebselen as a pharmaceutical agent has been extensively tested. Ebselen has been subjected to numerous structural modifications We thought it would be interesting to prepare Ebselen derivatives carrying a variety of *N*-substituents (nitro-, azo- and amino groups) in position 7. In Publication 2 we describe the preparation of nitro-Ebselens, bis-azo-Ebselens and diselenides carrying an amino group next to selenium. Whereas bis-azo-Ebselens were poor mimics of the GPx-enzymes, nitro-Ebselens and the diselenides were 3- to 6-fold more active than the parent as a catalyst for the decomposition of hydrogen peroxide. Based on ^{77}Se NMR spectroscopy, a mechanism was proposed for the reaction, involving amino-Ebselen as an intermediate. We also tested the protective effects of the best GPx-mimics in cellular systems. Human mononuclear cells (MNC) were stimulated to produce reactive oxygen species in the presence of antioxidants. As determined by chemiluminescence measurements, cytoprotection was greatly improved in the presence of some of the compounds.

In Publication 3 2,3-dihydrobenzo[b]selenophenes bearing an OH-group ortho, meta or para to selenium were prepared by a seleno-Claisen rearrangement followed by intramolecular hydroselenation. The radical-trapping activity and regenerability of the compounds were evaluated in a two-phase system in which linoleic acid was undergoing peroxidation in the lipid phase while regeneration of the antioxidant by various co-antioxidants (*N*-acetylcysteine, glutathione, dithiothreitol, ascorbic acid, tris-(carboxyethylphosphine)) was ongoing in the aqueous layer. The compound carrying the OH next to selenium quenched peroxyl radicals more efficiently than alfa-tocopherol. It also provided the most long-lasting antioxidant ptrotection. With thiol co-antioxidants it could inhibit peroxidation for more than five-fold longer than the natural product. An antioxidant mechanism involving O-atom transfer from peroxyl to selenium was proposed. The resulting phenolic selenoxide/alkoxyl radical would then exchange a hydrogen atom in a solvent cage before antioxidant regeneration at the aqueous lipid interphase.

2-Aryltellurophenols substituted in the aryltelluro or phenolic part of the molecule were prepared in Publication 4 by lithiation of the corresponding tetrahydropyran-protected 2-bromophenol, followed by reaction with a suitable diaryl ditelluride, then deprotection. In our two-phase system containing N-acetylcysteine as a co-antioxidant in the aqueous phase, all of the compounds quenched lipid peroxyl radicals more efficiently than alfa-tocopherol with three to five fold longer inhibition times. Compounds with electron donating substituents in the aryltelluro or phenolic part of the molecule showed the best results. The mechanism for quenching of peroxyl radicals was discussed in the light of calculated O-H bond-dissociation energies, deuterium labelling experiments and studies of thiol consumption in the aqueous phase.

Publications

PhD-thesis 1: “Exploring Catalytic Tellurium-Based Antioxidants” Jia-fei Poon, Uppsala University, **2016**.

PhD-thesis 2: “Regenerable Organochalcogen Antioxidants – An Explorative Study” Jiajie Yan, Uppsala University **2017**.

Publication 1. “Regenerable Radical-Trapping Tellurobistocopherol Antioxidants”. Poon, J.; Yan, J.; Singh, V. P.; Gates, P. J.; Engman, L. *J. Org. Chem.* **2016**, *81*, 12540-12544 (See Reprint 1).

Publication 2. “Nitro-, Azo-, and Amino Derivatives of Ebselen: Synthesis, Structure, and Cytoprotective Effects”. Singh, V. P.; Poon, J.; Yan, J.; Singh, V. P.; Lu, X.; Ott, M. K.; Butcher, R. J.; Gates, P. J.; Engman, L. *J. Org. Chem.* **2017**, *82*, 313-321 (See Reprint 2).

Publication 3. “2,3-Dihydrobenzo[b]selenophene Antioxidants: Proximity Effects and Regeneration Studies”. Singh, V. P.; Yan, J.; Poon, J.; Gates, P. J. ; Butcher, R. J.; Engman, L. *Chem. Eur. J.* **2017**, *23*, 15080-15088 (See Reprint 3).

Publication 4. “Substituent Effects in Aryltellurophenol Antioxidants”. Poon, J.; Yan, J.; Jorner, K.; Ottosson, H.; Donau, C.; Singh, V. P.; Gates, P. J.; Engman, L. *Chem. Eur. J.* **2018**, *24*, 3520-3527 (See Reprint 4).