
CHAPTER 8

Summary and general discussion

Many cells can reduce oxidized molecules that are present at their extracellular surface. The reducing equivalents that are required for these reactions originate from the intracellular space of the cell. The intracellular reductants, or electron donors, do not necessarily leave the cell to reduce extracellular substrates. Instead, the reducing equivalents, i.e. electrons, cross the membrane through a system in the plasma membrane. This thesis describes a study on the properties of these plasma membrane redox systems, and in particular their interaction with ascorbate (vitamin C). This vitamin plays an important role, both as an intracellular electron donor and as an extracellular substrate.

Ascorbate chemistry and detection

Ascorbate oxidizes in two single electron steps, yielding ascorbate free radical (AFR) and dehydroascorbic acid (DHA), respectively. DHA is not stable under physiological conditions, and will hydrolyze irreversibly. AFR is also short-lived in solution. For instance, two molecules of AFR can disproportionate, yielding one molecule of ascorbate and one of DHA. This disproportionation reaction is reversible, and will reach an equilibrium state. Thus, AFR can also be generated by mixing ascorbate and DHA. This principle has been used in the past to generate AFR in experiments. The resulting concentration of AFR was calculated from the equilibrium constant of the disproportionation reaction. Chapter 2 of this thesis discusses some drawbacks of this approach. AFR is not only formed by the disproportionation reaction, but also from the spontaneous or metal-ion-mediated oxidation of ascorbate. Especially at low, physiologically relevant, concentrations of ascorbate, this oxidation will dominate the formation of AFR. Thus, a calculation of the concentration of AFR using the equilibrium constant of the disproportionation reaction will underestimate the actual concentration. Moreover, the generation of AFR from ascorbate and DHA will not result in the prolonged and stable generation of AFR, as DHA will quickly hydrolyze, thereby changing the equilibrium. It is concluded that other methods should be used to generate AFR. A good alternative is the oxidation of ascorbate by the enzyme ascorbate oxidase, which gives a more stable and predictable concentration of AFR. Nevertheless, the resulting AFR concentrations should always be measured experimentally.

The dynamic chemistry of ascorbate makes it a challenging molecule to study. Ascorbate is subject to oxidation, and its oxidation products are even more labile. Therefore, techniques are required that prevent the degradation of the sample, or measurements must be made *in situ*. Chapter 7 describes some problems and solutions related to the analysis of ascorbate, DHA and AFR, and their interactions

in a cellular environment. A variety of techniques, such as UV spectroscopy, electron spin resonance spectroscopy, HPLC and radioactive labeling can be used to track the different molecules as they react and move between cells.

Ascorbate and plasma membrane redox systems

Ascorbate is known as an indispensable component of the anti-oxidant defenses. Many of its anti-oxidant effects involve a direct reaction with an oxidant. However, for some reactions, the interaction of ascorbate with plasma membrane redox systems is required. The transfer, by these systems, of reducing equivalents across the membrane allows intracellular ascorbate to reduce extracellular molecules without leaving the cell. The intracellular space has an efficient regeneration system for the intracellular oxidation products of ascorbate, ensuring efficient reduction back to ascorbate. It is still not completely clear what the physiological substrates and functions of the plasma membrane redox systems are. The most important function of the ascorbate-dependent redox systems appears to be protection against oxidants. Various potentially harmful oxidants can be reduced directly at the cell surface by the redox system. However, the reduction of extracellular AFR, and therefore the preservation of extracellular ascorbate levels, may be of more importance. After extracellular ascorbate reacts with an oxidant, it can be regenerated from its product AFR by a reduction at the cell surface. The use of a plasma membrane redox system for this reduction is more efficient than the intracellular regeneration of ascorbate, as it circumvents different transport steps, which delay the availability of the regenerated ascorbate to the extracellular medium.

Two important electron acceptors were chosen for further study in this thesis. Ferricyanide is a non-physiological oxidant that has frequently been used for the study of plasma membrane redox systems. Cells are impermeable to this molecule, and its conversion can easily be quantified experimentally. The other substrate that was studied was the ascorbate free radical. The conversion of this substrate to ascorbate is more difficult to assess, but the reduction of AFR is physiologically more important.

The importance of ascorbate to plasma membrane redox systems was revealed by the earlier study of ferricyanide reduction by erythrocytes (1-4). Accumulation of ascorbate in the cells increased the rate at which extracellular ferricyanide could be reduced. However, various models were proposed to explain this effect of ascorbate. In chapter 3, the ascorbate-dependent reduction of ferricyanide was

studied in the human leukemic cell line HL60. Indeed, intracellular ascorbate stimulated ferricyanide reduction in a saturable dose-dependent manner. Our data excluded several models, but corroborated a model in which ferricyanide is reduced via a plasma membrane redox system, similar to the reduction of ferricyanide by erythrocytes as described by May et al. (4). The cells can also reduce ferricyanide in the absence of ascorbate, with NADH as the intracellular electron donor. However, it was unknown whether different redox systems were involved in the reduction of ferricyanide by either ascorbate or NADH. Experiments with the inhibitor *p*CMBS revealed that the reduction of ferricyanide in the absence of ascorbate was much more sensitive to the inhibitor than the reduction in the presence of ascorbate. This indicates that separate redox systems must be present in the plasma membrane of HL60 cells, one that uses NADH as its electron source, and one that uses ascorbate.

Though ferricyanide is a useful tool for the study of plasma membrane redox systems, it is a non-physiological molecule. The physiological relevance of ferricyanide reduction is therefore not always clear. It has previously been shown that cells could also reduce extracellular AFR using intracellular NADH (5, 6). AFR could be a physiological substrate for plasma membrane redox systems. Therefore, experiments were conducted to study whether ascorbate-dependent redox systems could also reduce AFR. As described in chapter 4, this was indeed the case in erythrocytes. The cells could also reduce AFR using NADH, but intracellular ascorbate significantly increased the reduction rate. Thus, AFR seems to be a physiological substrate for the ascorbate-dependent plasma membrane redox system of the erythrocyte. The effect of ascorbate on the reduction of AFR was quite similar to its effect on the reduction of ferricyanide, as found in HL60 cells. It is therefore conceivable that a single protein is responsible for the reduction of both AFR and of ferricyanide. However, further experiments are required to test this hypothesis.

Mechanisms and components of ascorbate-dependent redox systems

Extensive evidence showed that intracellular ascorbate can promote the reduction of extracellular substrates. However, the exact mechanism behind this phenomenon has remained unclear. The extracellular substrates AFR and ferricyanide both require one electron for their reduction. It is therefore most likely that intracellular ascorbate will also donate one electron, yielding intracellular AFR. Indeed, the formation of intracellular AFR has been observed while extracellular AFR or ferricyanide was reduced ((7), this thesis). The NADH-dependent reduction of AFR and ferricyanide

must have a more complex mechanism. NADH donates two electrons, while the acceptors can only accept one. Hence, the NADH-dependent plasma membrane redox system must contain a buffer system to accommodate both one- and two-electron interactions. This may involve the participation of quinone or flavin moieties in the electron transfer mechanism.

The electrons that are donated by the intracellular reductant must be transported across the plasma membrane by a redox system. It has been suggested that small lipid soluble molecules like coenzyme Q and α -tocopherol could act as an electron shuttle to cross the plasma membrane (8-10). However, our data indicate that it is more likely that protein factors in the membrane are involved. Ascorbate-dependent redox systems displayed saturable kinetics in both HL60 cells and erythrocytes. Also, ferricyanide reduction in HL60 cells could be inhibited by thiol reactive agents, and depletion or supplementation of the cells with α -tocopherol did not affect reductase activity, nor did the addition of analogues of coenzyme Q. In erythrocytes, the experiments described in chapter 5 showed that the ascorbate-dependent reduction of AFR affected the membrane potential. Thus, the reduction was electrogenic, causing the net transport of charge across the membrane during the reaction. Coenzyme Q and α -tocopherol could potentially shuttle electrons across the membrane, but can only accept an electron together with a proton. Thus, for e.g. coenzyme Q, the possible redox forms are the neutral molecules Q, $\text{QH}^\bullet$, and QH_2 . A shuttle of coenzyme Q across the membrane can therefore not be electrogenic, whereas the electrogenicity of the reduction of AFR was clearly shown in chapter 5. For both erythrocytes and HL60 cells, a protein-mediated system is the best explanation for the data that were obtained on ascorbate-dependent redox systems. The involvement of a small electron carrier cannot be excluded for the NADH-dependent reduction of AFR or ferricyanide. Electrogenicity could not be shown under those conditions, whereas other groups provided evidence for a coenzyme Q shuttle that, driven by the NADH-dependent cytochrome b_5 reductase, could reduce extracellular AFR (9, 11). In spite of some opposing reports (12), the latter model seems a viable option to explain the NADH-dependent reduction of extracellular substrates.

The study of plasma membrane redox systems would be greatly facilitated by the identification of the putative proteins that drive the reaction. Comparison of expression levels in different tissues would be possible using molecular biological techniques, avoiding the problems that accompany functional assays. Also, it would be possible to resolve the questions that exist on the substrate specificity of the redox systems. At present, it is only possible to observe the conversion of substrates

by intact cells. It is therefore hard to establish whether multiple systems contribute to the conversion of a single substrate, or whether different substrates are converted by a single system. The ascorbate-dependent reduction of AFR in the erythrocyte strongly resembles a process in the adrenal gland. The adrenal medulla contains cells with chromaffin granules, in which large amounts of ascorbate are oxidized to AFR for the biosynthesis of norepinephrin. This AFR is subsequently reduced by a cytochrome b_{561} in the granule membrane, which uses cytoplasmic ascorbate as an electron donor. It was hypothesized that this cytochrome, which had never been found in the erythrocyte, might be responsible for the same reaction in the erythrocyte membrane. Therefore, the expression of cytochrome b_{561} was studied in erythrocyte membrane extracts with an antibody against cytochrome b_{561} , and by RT-PCR in mRNA from reticulocytes, erythrocyte progenitor cells that still contain nucleic acids (Chapter 6). However, no evidence could be found for cytochrome b_{561} using either technique. Thus, cytochrome b_{561} cannot be the ascorbate:AFR reductase of the erythrocyte. It is thought that another, possibly similar, protein is present in the erythrocyte for the ascorbate-dependent reduction of AFR.

Future attempts to identify the erythrocyte protein could start on the presumption that it has structural similarity to cytochrome b_{561} . Though this is by no means certain, it allows for a number of practical search strategies that have a good chance of success. The spectral properties of cytochromes could be exploited to find unknown cytochromes in the erythrocyte membrane, that have a midpoint potential which is appropriate for redox reactions with ascorbate. Alternatively, it could be attempted to find genes or proteins that have a sequence homology with cytochrome b_{561} . If the erythrocyte protein is related to cytochrome b_{561} , crucial segments of its structure, like heme or ascorbate binding sites, are likely to be conserved. Similar domains can be sought in the human genomic databases that are rapidly being developed by private and public institutes. Subsequently, the expression of candidate genes could be tested in the erythrocyte. The identification of a plasma membrane reductase will be more difficult when it is unrelated to cytochrome b_{561} . Possibly, a cDNA library of erythrocyte precursor cells can be screened for features of other membrane redox proteins. However, it is not certain that the ascorbate:AFR reductase of the erythrocyte is still transcribed in the reticulocyte stage. It appears difficult to screen isolated protein fractions from erythrocytes for the desired activity, as this would require the proper reconstitution of the protein in a sealed membrane environment. With any of the strategies that were mentioned, it seems that considerable effort is still required to identify the ascorbate-dependent reductase(s) in the plasma membrane.

Physiological relevance and distribution

The experiments described in this thesis unavoidably do not reflect the physiological conditions that can be expected *in vivo*. Ascorbate loading yielded a concentration of 1 mM in erythrocytes, whereas 50 μ M is the normal level. Extracellular AFR concentrations were probably higher than under normal oxidative stress, while ferricyanide is not physiological at any concentration. High concentrations were required to reveal the properties of a system, also due to the limitations of the techniques that were used. Nevertheless, the results do bear a significant relevance to the physiological situation. Systems that are characterized using high levels of substrate are likely to function at lower concentrations as well. Indeed, the data indicated that ascorbate-dependent redox systems must still have significant effects at an intracellular concentration of 50 μ M. Also, it must be noted that other cells, such as neutrophils, can contain ascorbate up to the millimolar range. However, little is known about plasma membrane redox systems in such cells.

Throughout this thesis, blood-borne cells were used as a model. Blood is exposed to relatively high oxidative stress, among others due to its high oxygenation. Assuming that plasma membrane redox systems are involved in the protection against extracellular oxidants, blood is therefore a likely place to find these systems. Ascorbate-dependent redox systems have not been studied in many other tissues. Evidence has been found in endothelial cells of the pulmonary artery and in perfused rat liver, but also e.g. in plants (13-15). Thus, the evidence for an ascorbate-dependent redox system is available for only a limited number of cell-types.

In summary, the results that were presented show that both erythrocytes and HL60 cells contain plasma membrane redox systems that can reduce extracellular substrates. Intracellularly, either NADH or ascorbate can donate electrons to drive these reactions. It was found in HL60 cells that, though both NADH and ascorbate could drive the reduction of extracellular ferricyanide, separate systems must be present in the plasma membrane of the cells to transfer the reducing equivalents. Also, the results showed that it is unlikely that α -tocopherol or coenzyme Q are involved in these systems. A physiological extracellular substrate of plasma membrane redox systems is AFR. This substrate was reduced by erythrocytes, depending on either intracellular NADH or intracellular ascorbate. The ascorbate-dependent reduction of AFR was electrogenic, confirming vectorial electron transport across the plasma membrane. Also, the electrogenicity of the reaction implies that ascorbate-dependent electron transfer is not mediated by the diffusion of small electron carriers like coenzyme Q. Instead, it is likely that a membrane protein is

involved. Cytochrome b_{561} could potentially explain the effect, but was found to be absent in the erythrocyte. Instead, it is possible that homologues of this cytochrome in the erythrocyte membrane are responsible for the ascorbate-dependent redox reactions of this cell.

References

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Samenvatting voor de leek

In dit proefschrift wordt een redoxsysteem beschreven dat in de plasmamembraan (buitenwand) van de meeste cellen aanwezig lijkt te zijn. Het systeem zorgt voor het omzetten van stoffen aan de buitenkant van de cel via een chemische reactie waarbij elektronen worden overgedragen, een zogenaamde redox reactie. De voor deze reactie benodigde elektronen worden aan de binnenkant van de cel afgegeven door een donor. Het redoxsysteem zorgt ervoor dat de elektronen over de plasmamembraan worden vervoerd naar de buitenkant van de cel, en daar worden gebruikt om een acceptormolecuul om te zetten. Hoewel er verschillende ideeën bestaan over het doel van deze reacties, lijken ze belangrijk te zijn bij het tegengaan van oxidatieve stoffen. Deze stoffen -onder meer radicalen- zijn erg reactief, en kunnen essentiële onderdelen van de cel beschadigen. Dit kan worden voorkomen door de oxidatieve stoffen snel onschadelijk te maken, voordat ze iets beschadigen. Mogelijk spelen de redoxsystemen in de plasmamembraan hierbij een rol.

Ook ascorbaat, beter bekend als vitamine C, maakt oxidatieve verbindingen onschadelijk met een redoxreactie. In deze studie is gebleken dat ascorbaat en de redoxsystemen kunnen samenwerken om dit nog beter te doen. Een belangrijke reden voor de samenwerking is de regeneratie van ascorbaat. Deze vitamine kan worden vergeleken met een oplaadbare batterij. Na de reactie met een oxidatieve verbinding is de batterij leeg, en nutteloos voor het lichaam. Het is dan ook belangrijk dat de batterij snel weer opgeladen wordt. Het opladen van de batterij gaat het beste aan de binnenkant van de cel. Het redoxsysteem kan elektronen van ascorbaat binnen de cel doorgeven aan oxidatieve verbindingen buiten de cel. Het dient zo als het ware als een verlengsnoer om de elektronen van ascorbaat naar buiten te brengen. Op deze manier helpt ascorbaat bij het omzetten van stoffen buiten de cel, maar kan zelf binnen blijven en efficiënt geregenereerd worden.

Toch is de aanwezigheid van ascorbaat ook buiten de cel noodzakelijk, en moet het ook daar na een reactie geregenereerd kunnen worden. We hebben gevonden dat het regenereren buiten de cel plaats kan vinden met behulp van het redoxsysteem in de plasmamembraan, en andere ascorbaat moleculen die aan de binnenkant van de cel aanwezig zijn. Dit systeem kan vergeleken worden met een lege batterij aan de buitenkant van de cel die, via een verlengsnoer, opgeladen wordt met behulp van een volle batterij aan de binnenkant van de cel. Zo wordt er voor gezorgd dat er, ook aan de buitenkant van de cel, altijd voldoende ascorbaat aanwezig is om het lichaam te beschermen.

We hebben ook geprobeerd uit te vinden hoe het systeem in de celwand is opgebouwd. De reacties aan de buitenkant van de cel lijken veel op die van een speciaal eiwit dat van een andere plaats in het lichaam bekend was. Er is getest of dit eiwit ook voorkwam in de plasmamembraan van de cellen die wij bestudeerd hebben. Dit bleek echter niet het geval te zijn. Wel bleek uit veel kenmerken van de reacties van het systeem dat er waarschijnlijk een ander, nog onbekend eiwit bij betrokken moet zijn. Het is goed mogelijk dat het nieuwe eiwit een beetje op het reeds bekende eiwit lijkt. Dat laatste zou kunnen helpen om het later te kunnen vinden.

List of publications

M.M. Van Duijn, J. Van der Zee, J. VanSteveninck and P.J.A. Van den Broek. Ascorbate stimulates ferricyanide reduction in HL-60 cells through a mechanism distinct from the NADH-dependent plasma membrane reductase. *J Biol Chem* 1998; 273:13415-13420.

M.M. Van Duijn, J. Van der Zee and P.J.A. Van den Broek. Electron spin resonance study on the formation of ascorbate free radical from ascorbate: the effect of dehydroascorbic acid and ferricyanide. *Protoplasma* 1998; 205:122-128.

M.M. VanDuijn, K. Tijssen, J. VanSteveninck, P.J.A. Van den Broek and J. Van der Zee. Erythrocytes reduce extracellular ascorbate free radicals using intracellular ascorbate as an electron donor. *J Biol Chem* 2000; 275:27720-27725.

M.M. VanDuijn, J.T. Buijs, J. Van der Zee and P.J.A. Van den Broek. The ascorbate:AFR oxidoreductase from the erythrocyte membrane is not cytochrome b_{561} . *Protoplasma*; In Press.

M.M. VanDuijn, J. Van der Zee and P.J.A. Van den Broek. The ascorbate dependent reduction of extracellular ascorbate free radicals by the erythrocyte is an electrogenic process. *FEBS Lett* 2001; 491:67-70.

M.M. VanDuijn, J. Van der Zee and P.J.A. Van den Broek. Interaction of extracellular and intracellular ascorbate: detection and characterization. Submitted for publication in *Methods Enzymol*.

Curriculum Vitae

Martijn Maurice van Duijn werd op 16 augustus 1972 geboren in Katwijk. Na het behalen van het diploma in het Voorbereidend Wetenschappelijk Onderwijs aan het Pieter Groen College in Katwijk, werd in 1990 aan de Universiteit Leiden aangevangen met de studie scheikunde. Nadat een jaar later de propaedeuse was behaald, is de studie voortgezet in de bovenbouwstudie Bio-Farmaceutische Wetenschappen. Deze studie werd afgerond met een tweetal stages. De eerste betrof een onderzoek aan de foto-isomerisatie van urocaanzuur, een proces dat samenhangt met UV-geïnduceerde immuunsuppressie. Dit onderzoek aan de afdeling Medicinale Fotochemie, onder Prof. Dr. G.M.J. Beijersbergen van Henegouwen, werd na een jaar voltooid, en werd gevolgd door acht maanden onderzoek aan de afdeling Dermatology, van het Massachusetts General Hospital in Boston, USA. Onder Dr. T. Hasan werd het effect van fotodynamische therapie op signaal-transductie door de EGF receptor onderzocht. Na deze stages werd in september 1996 het doctoraal diploma in de Bio-Farmaceutische Wetenschappen behaald.

In december 1996 is begonnen met het promotieproject over plasma membraan redox systemen in de vakgroep Moleculaire Cel Biologie van het Leids Universitair Medisch Centrum. Dit onderzoek werd begeleid door Dr. P.J.A. van den Broek en Dr. J. van der Zee, onder supervisie van Prof. Dr. J. van Steveninck. Na diens overlijden in 1998 is de supervisie overgenomen door Prof. Dr. H.J. Tanke. Tot december 2000 zijn in deze groep de werkzaamheden uitgevoerd die hebben geleid tot dit proefschrift.

Na zijn promotieonderzoek heeft de auteur een post-doctorale onderzoeksfunctie aanvaard aan het FOM instituut voor Atoom- en Molekuulfysica in Amsterdam.

Nawoord

Hoewel mijn naam als enige voor op dit boekje staat, is het natuurlijk absoluut onjuist dat de totstandkoming ervan de verdienste van één persoon zou zijn. Vele mensen hebben een bijdrage geleverd in de afgelopen jaren, en dit is de plek om hen daarvoor te bedanken.

Om te beginnen is er Karmi, die tijdens het gehele project hulp heeft geboden als analiste, en daarbij veel geduld heeft getoond. Ook Laurence heeft me enige tijd als analiste geholpen, maar werkt inmiddels als AIO aan haar eigen promotie. Verder hebben Verena, Marissa en Jeroen zich als stage-studenten enige maanden willen inspannen voor mijn project. Ook zij waren een grote hulp, zowel door hun praktische werk, als door de discussie over het werk die een stage-project met zich meebrengt. Helaas hebben niet al hun projecten kunnen uitgroeien tot een afgerond geheel in dit proefschrift, maar dat heeft zeker niet aan hun inzet gelegen. Ook dank aan mijn familie, voor de basis die ze voor mij hebben gelegd, en voor de niet-aflatende steun die ze bieden. Verder wil ik alle andere vrienden en collega's die mij in woord en daad hebben bijgestaan de afgelopen jaren bedanken voor hun onmisbare bijdrage.

De laatste vermelding is altijd voor de belangrijkste persoon. Liefvie, in de jaren dat dit proefschrift tot stand is gekomen hebben we nog heel wat meer gebouwd, en je hebt je op die manier aardig onmisbaar weten te maken. Bedankt voor alles!

Martijn

Stellingen

Deze stellingen behoren bij het proefschrift "Ascorbate and its interaction with plasma membrane redox systems"

1. De reductie van extracellulaire ascorbaat radicalen door ascorbaat-afhankelijke plasmamembraan redoxsystemen levert een fysiologisch belangrijke bijdrage aan de instandhouding van de extracellulaire ascorbaat concentratie.

Dit proefschrift.

2. Aangezien ascorbaat en NADH een verschillend aantal electronen overdragen in een redoxreactie, is te verwachten dat de plasmamembraan redoxsystemen waaraan zij electronen doneren volgens verschillende mechanismen werken, en dat de betrokken eiwitten weinig homologie zullen vertonen.

Dit proefschrift.

3. Het feit dat de zogenaamde disproportioneringsreactie resulteert in een evenwicht tussen ascorbaat radicalen en ascorbaat en dehydroascorbaat, maakt het experimenteel aantonen van interconversie tussen ascorbaat en dehydroascorbaat een voorbeeld van het opnieuw uitvinden van het wiel.

Dit proefschrift,

Nishikawa Y, Kurata T. Interconversion between dehydro-L-ascorbic acid and L-ascorbic acid. *Biosci Biotechnol Biochem* 2000; 64:476-483.

4. Het nalaten van een bepaling van de NADH-concentratie in de cel na depletie van ascorbaat met behulp van TEMPOL belemmert het trekken van eenduidige conclusies over de betrokkenheid van ascorbaat bij cellulaire redoxreacties.

Dit proefschrift,

May JM, Qu ZC et al. Protection and recycling of α -tocopherol in human erythrocytes by intracellular ascorbic acid. *Arch Biochem Biophys* 1998; 349:281-289,

Winkler BS, Orselli SM et al. The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. *Free Radic Biol Med* 1994; 17:333-349).

5. De aanname dat remmers van glucosetransport de opname van dehydroascorbaat kunnen voorkomen is niet van toepassing op een experiment waarbij erythrocyten worden gebruikt.

Dit proefschrift,

Himmelreich U, Kuchel PW. C-13-NMR studies of transmembrane electron transfer to extracellular ferricyanide in human erythrocytes. *Eur J Biochem* 1997; 246:638-645.

6. Aangezien de consumptie van hoge doses ascorbaat resulteert in een snelle uitscheiding in de urine, is van het gebruik van dergelijke doses weinig extra bescherming te verwachten.
7. Hoewel het vaststellen van de aminozuurvolgorde van eiwitten over het algemeen weinig problemen oplevert, zijn voorspellingen over de ruimtelijke structuur, wanneer niet ondersteund door technieken als röntgen-diffractie of cryo-EM, zodanig afhankelijk van de interpretatie van de onderzoeker dat er vaak sprake is van niet meer dan een 'educated guess'.
 Fleming PJ, Kent UM. Cytochrome b_{561} , ascorbic acid, and transmembrane electron transfer. *Am J Clin Nutr* 1991; 54:1173s-1178s,
 Srivastava M, Gibson KR et al. Human cytochrome b_{561} : a revised hypothesis for conformation in membranes which reconciles sequence and functional information. *Biochem J* 1994; 303:915-921.
8. De aanwezigheid van evenveel publicaties over stimulerende dan wel remmende effecten van ascorbaat op de celproliferatie suggereert dat er geen direct verband is tussen ascorbaat en proliferatie.
 Alcain FJ, Buron MI et al. Ascorbate free radical stimulates the growth of a human promyelocytic leukemia cell line. *Cancer Res* 1990; 50:5887-5891,
 Park CH, Kimler BF. Growth modulation of human leukemic, preleukemic, and myeloma progenitor cells by L-ascorbic acid. *Am J Clin Nutr* 1991; 54:1241s-1246s.
9. Omdat reactieve zuurstofvormen belangrijke functies vervullen in normale fysiologische processen, kan overmatige consumptie van anti-oxidanten, bijvoorbeeld in de vorm van voedingssupplementen, leiden tot ongewenste neveneffecten.
10. Doordat werkzaamheden in toenemende mate afhankelijk zijn van informatietechnologie, is het noodzakelijk geworden dat er in de werkomgeving minstens één werknemer aanwezig is, die in staat is de dagelijkse computerproblemen van de rest op te lossen.
11. De prestaties van de Nederlandse en andere spoorwegen doen het gezegde 'Het loopt als een trein' aan betekenis inboeten.
12. Analyses met een HPLC-systeem geven de wetenschapper een hernieuwde waardering voor het oude ambacht van loodgieter.
13. Het wereldwijde milieubeleid doet vermoeden dat de uitstoot van CO₂ pas significant zal gaan dalen wanneer de voorraad fossiele brandstof uitgeput raakt.

Martijn van Duijn

Ascorbate and its interaction with plasma membrane redox systems

Many cells contain a redox system in their plasma membrane that can mediate the transfer of electrons from intracellular donors to extracellular acceptors. Several functions have been attributed to the system, but its most important function seems to be the protection against oxidants. This is also reflected in its interaction with the important anti-oxidant molecule ascorbate, or vitamin C.

This thesis describes several studies on ascorbate chemistry, plasma membrane redox systems, and, in particular, their interaction with ascorbate. It was found that separate redox systems exist in the plasma membrane, which can use either NADH or ascorbate as intracellular electron donors for the reduction of extracellular substrates. Other experiments showed that plasma membrane redox systems also interact with extracellular ascorbate. An oxidation product of ascorbate, the ascorbate free radical, was reduced on the extracellular face of the cell by a redox system that uses intracellular ascorbate as an electron donor. Thus, extracellular ascorbate is regenerated, and loss of the vitamin is prevented. The ascorbate free radical is believed to be an important physiological substrate for plasma membrane redox systems.

The components of plasma membrane redox systems are still unknown. The involvement of one candidate membrane protein in the ascorbate-dependent redox reactions was excluded. Nevertheless, the experiments strongly suggest that a protein-mediated mechanism must be involved, rather than alternative mechanisms, such as a shuttle of small lipid soluble molecules like α -tocopherol.

Regardless of the mechanism involved, the plasma membrane redox systems described in this thesis seem to make a significant contribution to the anti-oxidant effects of ascorbate.

