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Introduction

Analysis of the L-arginine/NO pathway☆

In 1987, the scientific community learned that the human body produces nitric oxide (NO). The underlying unique mechanism of NO production involves oxidation of the imino group of L-arginine to NO using molecular oxygen as a co-substrate and formation of L-citrulline as the second reaction product. This discovery was a surprise in so far that NO had been previously known as a harmful component of exhaust fumes. Encouraged by this finding, the endothelium-derived relaxing factor (EDRF), discovered beforehand in 1980, could be identified as NO or a labile NO-containing species. NO is a free-diffusible radical gas and possesses high affinity to haem-containing proteins, notably soluble guanylyl cyclase and oxyhaemoglobin, and to other radicals, notably the superoxide radical anion ($O_2^{\bullet -}$). These characteristic physicochemical properties are primarily responsible for the multiple biological actions of NO as well as for its complex metabolic fate. The recognition of the tremendous importance of NO in human circulation was honoured by the Nobel Prize for medicine in 1998 and before that NO was crowned by the scientific community as the molecule of the year 1992. Today, it is hard to imagine biomedical and life sciences without the L-arginine/NO pathway.

In the past 20 years, researchers from various disciplines put a lot of effort into a better understanding of this very promising but labyrinthine and highly challenging pathway. Our knowledge grew enormously but it still remains fragmentary. Despite considerable success in recent years the L-arginine/NO pathway is far from being delineated, and so far humanity has taken insufficient advantage of its enormous potential. A possible explanation for this lies in the complexity of this pathway which poses a big challenge to analytical chemistry. By nature, the quality of our scientific results and consequently of our knowledge is fully dependent on the quality of the analytical methods used to achieve them. For this reason, analytical chemistry is generally of vital importance to generate solid knowledge and real progress. This especially applies to the L-arginine/NO pathway which is frequently characterized as an analytical minefield. In consideration of the exceptional importance of analytical chemistry in biomedical and life sciences, this special issue is dedicated to the *Analysis of the L-arginine/NO pathway*.

The L-arginine/NO pathway comprises numerous members belonging to distinctly different classes of low-molecular mass and high-molecular mass substances. Important inorganic members of this family are NO and its oxidative metabolites nitrite and nitrate, its reductive metabolite nitroxyl (NO^-), as well as peroxynitrite ($ONOO^-$), i.e. the intermediate product formed from the reaction of NO with ubiquitous $O_2^{\bullet -}$. Primary organic members of this pathway include L-citrulline, L-arginine and its N^G-methylated analogues asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and monomethylarginine (L-NMMA), as well as various cofactors, notably tetrahydrobiopterin. Important enzymes of this pathway are NO synthase (NOS), proteinmethyltransferases that produce ADMA, dimethylarginine dimethylaminohydrolases (DDAH) which hydrolyze ADMA to L-citrulline and dimethylamine (DMA), and the NO target enzyme soluble guanylyl cyclase (sGC), with its product cGMP being the second messenger of NO. NO itself and other short-lived metabolites of NO such as peroxynitrite are not accessible to direct analysis. However, formation of such species can be traced by measuring stable metabolites such as nitrite and nitrate, 3-nitrotyrosine and *S*-nitrosothiols (RSNO), with the latter even exerting potent NO-related bioactivity.

This special issue contains peer-refereed review articles and research papers. Because of the great variety of analytes not all known members of the L-arginine/NO pathway could be considered in this work. The review articles were selected based on the following criteria: (1) accessibility to analytical quantitative determination; (2) NO-related bioactivity or function as a measure of enzyme activity, notably of NOS and DDAH; (3) clinical relevance, i.e. quantitative analysis in human biological fluids in health, disease and during pharmacological intervention or physical exercise. In addition, genomics- and proteomics-related issues of the L-arginine/NO pathway were also considered in review articles due to increasing importance and relevance to the present.

The review articles in this special issue were written by scientific experts in these particular fields. These articles focused on the analysis of: (1) circulating nitrite as a measure of endothelial NO synthesis as well as a storage form of NO in the circulation; (2) circulating and urinary nitrate as a measure of systemic and whole body NO synthesis respectively; (3) *S*-nitrosothiols both as species with NO-related actions and a storage form of NO

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in the circulation; (4) ADMA and other methylated L-arginine analogues as endogenous inhibitors of NO synthesis; and (5) 3-nitrotyrosine as a footprint left by peroxynitrite and other reactive nitrogen species. The irony of this selection mode is that authentic NO, the half-life of which in human circulation is considerably shorter than 1 s, was not considered as a primary real analyte in this work. Nevertheless, the current state of the particular technology of electron paramagnetic resonance (EPR) spin trapping for experimental and clinical NO biology was reviewed. The research papers included in the special issue *Analysis of the* L-*arginine/NO pathway* dealt with new approaches suitable to measure DDAH activity in tissue in vitro and in vivo as well as with improvement of existing methods for the analysis of nitrite, nitrate and 3-nitrotyrosine.

The review articles revealed a wealth of ideas of scientists which provided a plethora of analytical methods and techniques to measure in principle every member of the L-arginine/NO pathway including NO itself. These methodologies include the famous spectrophotometric Griess reaction, fluorimetry, chemiluminescence, flow injection analysis (FIA), HPLC, CE, GC–MS, GC–MS/MS, LC–MS/MS, and more recently ELISA for ADMA. Successful combinations of particular techniques have also recently been reported, especially the coupling of HPLC with the Griess reaction or chemiluminescence as the detection mode for nitrite measurement. Recent advances in particular technologies and in automation now make possible high throughput analysis of important members of the L-arginine/NO family, notably nitrite and ADMA. In particular the great improvement achieved in recent years in the LC–MS/MS technique enables high throughput analysis within a run of several members, e.g. L-arginine, ADMA and SDMA, without renouncing accuracy and precision. The great analytical potential of the LC–MS/MS approach in terms of accuracy, sensitivity, versatility and high throughput analysis seems to increasingly gain ground towards the "classical" HPLC and GC–MS techniques. Nevertheless, in particular MS-based technologies are still limited to a few investigator groups. By far the majority of the researchers give priority to batch spectrophotometric and fluorimetric assays.

In the review articles, the authors paid special attention to specific analytical problems associated with the analysis of certain members of the L-arginine/NO pathway with a particular methodology. Analytical methods for those particular analytes were discussed from several points of view. Apparently, many of the analytical problems are largely unrelated with the methods used and possess general validity. This especially applies to nitrite which is ubiquitous. Other analytical problems are closely related to the methods used. This has been exemplified for *S*-nitrosothiols and 3-nitrotyrosine which can be artificially formed during the various steps of the experimental procedures used. These analytical shortcomings seem to be responsible for the great discrepancy concerning physiological concentrations of *S*-nitrosothiols and 3-nitrotyrosine in particular, and to a lesser extent to nitrite. So far, these members of the L-arginine/pathway evaded the generally accepted definition of reference values and intervals. By contrast, almost all available techniques provide comparable physiological ADMA concentrations in the human circulation, suggesting that currently available analytical methods possess comparable analytical performance and reliability. Another special analytical issue discussed by review authors is the insufficient and some times even missing validation of methods used for quantitative measurement in relevant biological fluids. In this context, commercial availability of "ready-to-use" assays including the enzymatic batch Griess assay and the HPLC–Griess method is very tempting. Also, because of numerous analytical shortcomings comparison of methods for a particular member of the L-arginine/NO family as well as evaluation of the state of this pathway in clinical studies are very difficult.

Forty-eight of the most active analytically oriented researchers in this field were invited to contribute articles to this special issue. Twenty-four of these were able to accept the invitation resulting in a total of 27 submissions and the final complement of the papers included herewith. I thank all authors for their contribution to the special issue *Analysis of the* L-*arginine/NO pathway* and their fruitful discussion during the review process. Scientific experts in particular fields of the L-arginine/NO pathway served as objective, unselfish and vigilant referees of the papers included in this special issue. These scientists are sincerely thanked for their essential work and contribution. I also thank all those scientists who were not able to accept the invitation to write articles for this special issue but obligingly offered their services as reviewers or editorial advisers.

Authors, reviewers and guest editor trust this special issue will inform the reader about the methods of analysis currently available for relevant members of the L-arginine/NO pathway, will sensitize both experienced scientists and inexperienced young researchers to the exceptional importance of analytical chemistry in the area of NO research, and will also stimulate new research and development of new analytical methods in this field.

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