Albert Szent-Györgyi: vitamin C identification

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Today, our nutritional need for vitamin C (ascorbic acid) is well known and understood by the general public. As a dietary supplement, it is the vitamin with the largest commercial volume; some 10^8 kg (>US$600 million in the global market) are sold each year as tablets, a component of multivitamin products and an addition to many foods and drinks with the intention of promoting health. However, it can also be added to products simply to enhance the sales appeal. It can appear in unexpected places, such as chewing gum and sweets, personal care products and even in pet and animal feed (ascorbic acid is not considered to be a vitamin for animals other than humans). The demand for vitamin C is growing fast and new production facilities that are coming online in China are restructuring the manufacturing and supply of this product.

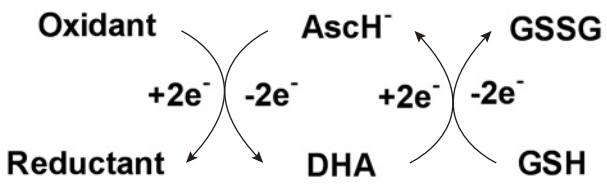
The isolation and identification of ascorbic acid as vitamin C was one of the most important advances that lead to improving human nutrition in the 20th Century. The delicate and seemingly fickle nature of this versatile reducing agent was one of the reasons that its isolation and the subsequent identification of its chemical nature presented such a major challenge. Professor Albert Szent-Györgyi was a key contributor to all aspects of the research that lead to the identification of vitamin C in the 1920s and 1930s. The *Biochemical Journal* published four key papers[1–4] from his laboratory, and these provided a wide range of insights into the basic chemistry of ascorbic acid and its identity as vitamin C, as well as the large-scale isolation of vitamin C from Hungarian red peppers[4].

By 1928, it was known that the adrenal cortex was somehow connected to biological oxidations; however, the nature of this connection could not be found. Szent-Györgyi found this connection by studying the juice of turnips, the roots of which contain significant quantities of the enzyme peroxidase. Peroxidase reduces hydrogen peroxide to water and in turn oxidizes an appropriate substrate. He demonstrated that there was a previously unrecognized reducing factor in the juice and that it was the preferred reducing agent for the enzyme. Other substrates would not be oxidized until this reducing factor was exhausted. He went on to demonstrate that a reducing factor that behaved in a similar fashion was present in onion, leek, cabbage, orange, lemon, grapefruit and apple.

Szent-Györgyi then demonstrated that the adrenal gland has a high level of a reducing factor with these same properties. He devised a method to isolate this substance and, using kilogram quantities of gland, was able to obtain relatively pure crystals of the 'reducing factor' (approximately 300 mg/kg of gland). He was the able to begin the characterization of this substance. In his

"Discovery consists of seeing what everybody has seen and thinking what nobody has thought".

Professor Albert Szent-Györgyi. Reproduced with the permission of the Nobel Foundation.



The recycling of vitamin C as discovered by Professor Szent-Györgyi. One of the many important discoveries presented in the 1928 paper[1] was that the reversible oxidation product of ascorbate could be reduced by glutathione. This concept was new and could explain how so little of the vitamin could do so much in an organism.

first paper in *The Biochemical Journal*[1] he reported the following properties of this reducing factor:

1. It is highly oxidizable.
2. The oxidation is catalysed by OH^- as well as metals such as iron and copper.
3. It has an acidic hydrogen atom and an equivalent mass of 178.
4. Its molecular mass is 178±3;
5. It appeared to be a lactone with the chemical formula $C_6H_8O_6$ and was given the name hexuronic acid*.
6. Hexuronic acid could be isolated from plants (e.g. oranges and cabbage), linking the observations on the reducing factor in plants and animals.
7. It is oxidized both reversibly and irreversibly.
8. It provides two hydrogen atoms (two equivalents) as a reversible reducing agent.
9. The reversible oxidation product of hexuronic acid is reduced by glutathione and other thiols.
10. The reversible oxidation product is 'intensely' reduced by animal tissues.

This paper[1] laid the foundation for much of the chemistry and biochemistry of vitamin C that we know today.

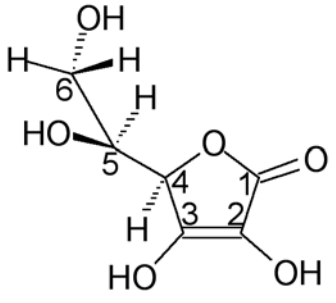
Szent-Györgyi had only a limited supply of hexuronic acid, as the isolation from adrenal glands was arduous and produced only milligram amounts of the substance. Nonetheless, enough was available for a test of its antiscorbutic (anti-scruvy) properties using guinea pigs. He found that hexuronic acid (1 mg/day) provided complete protection against scurvy and, indeed, that hexuronic acid is vitamin C[2,3]. He shared this large supply with all researchers working on vitamin C, including the leading carbohydrate chemist of that era, Walter Norman Haworth at the University of Birmingham. In Haworth's laboratory the definitive determination of this substance's structure was accomplished. To confirm the structure, the compound was also synthesized; this was the first artificial synthesis of a vitamin. It is interesting to note that the last line of the Banga and Szent-Györgyi paper[4] "No patents were taken out for the process."

With this large supply it was then possible to obtain a very pure, single-component preparation of hexuronic acid. This allowed a definitive animal test to be undertaken and indeed the antiscorbutic properties were found to be due to hexuronic acid, not to any possible contaminant in the preparation from adrenal glands.

With the definitive structure and the solid evidence that hexuronic acid was vitamin C, Szent-Györgyi and Haworth re-named it "a-scorbic" acid, because it prevented scorbutus (scurvy).

The 1937 Nobel committee honoured discoveries about vitamins. The Nobel Prize for Physiology or Medicine was awarded to Szent-Györgyi "for his discoveries in connection with the biological combustion processes, with especial reference to vitamin C and the catalysis of fumaric acid." Norman Haworth and Paul Karrer, shared that year's prize in chemistry. Haworth was awarded for his work on carbohydrates because "he has, above all, made clear the chemical structure of vitamin C" and Karrer "for brilliant investigations on carotenoids and flavins, as well as on vitamins A and B_2".

Many laboratories at that time worked on the isolation and identification of vitamin C, but it is



The structure of ascorbic acid.

*This work was done at Cambridge upon the invitation Sir Frederick Gowland Hopkins, the eminent British biochemist. It was Hopkins who urged him to publish these results in the *Biochemical Journal*. This required that the substance be named. Szent-Györgyi jokingly suggested calling it "Ignose" — from 'ignosco' (I don't know) and '-ose' to indicate a sugar. However, the editor rejected this suggestion as well as Szent-Györgyi's second choice, 'Godnose'. The editor proposed 'hexuronic acid' (using 'hex' to indicate the six carbon atoms). The University of Cambridge awarded Szent-Györgyi a PhD in biochemistry at the end of 1927 for this work.

clear that the first isolation and characterization of ascorbic acid during Szent-Györgyi's doctoral research paved the way for much of the progress made by the research community. His large-scale isolation of the compound and his sharing of it with other researchers was an incredible catalyst for fast progress in the understanding of this fickle vitamin.

Some 75 years later, vitamin C still offers many challenges to researchers. We still struggle with its instability, are learning the details of its biochemical functions, are dealing with controversy because we do not understand fully its pro- and anti-oxidant actions, as well as all of its biochemical functions and are learning new aspects of its potential medical applications.

It will continue to be a subject of controversy and research for many years to come and many of its mysteries remain to be uncovered. These classic papers in the *Biochemical Journal*[1–4] provided the foundation for the research on vitamin C in the 1920s and 1930s[5–7], as well as for continuing work in the modern era[8–13].

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The Albert Lasker Award 1954 presented by the American Heart Association to Albert Szent-Györgyi.

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Websites that have excellent further reading on the life and career of Professor Albert Szent-Györgyi and vitamin C

- The Nobel Prize website:http://nobelprize.org/medicine/laureates/1937/
- The National Institutes of Health profile of Professor Szent-Györgyi:http://profiles.nlm.nih.gov/WG/
- An excellent overview of the historical aspects of vitamin C:http://www.cambridge.org/us/books/kiple/vitaminc.htm