GLUCOSINOLATES NUTRACEUTICAL PRODUCTS ?

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Introduction

The absence of some essential nutrients and the presence of xenobiotics in our diet can produce or enhance a certain susceptibility to illness. In addition, many components of the human diet previously overlooked are now considered to play an important role in the prevention and treatment of important diseases. Thus over the last decade disease-preventing foods and ingredients, today called nutraceuticals, have met with great success. Nutraceutical is a word recently coined to transmit immediately the idea of something with properties intermediate between food and drug.

Many authors define nutraceutical as a food or any part of food that gives a health benefit above and beyond providing simple nutrition or basic fortification. This generally accepted definition establishes that health benefit may include not only the prevention or treatment of disease, but also a simple improvement in the body's performance. Examples include essentially herbs, fruits, microbes and minerals.

Today the most important nutraceutical formulations contain vitamins with antioxidant properties, oligosaccharides, sulfides, lactoferrin, omega-3 fatty acids, medium chain triglycerides, carotinoids, phenolic compounds of plant origin, terpens, and glucosinolates and/or their degradation products.

Nutraceuticals are products with the fastest growing market slice in the food and diet supplement industries. Over the last decade, the world sales of nutraceuticals have increased dramatically with an annual growth rate of about 20%. Without considering diet supplements, sugars and fat substitutes, fibre-enriched foods, fatless meat, skimmed milk, low calorie diets etc., this market in the USA is estimated at around $20 billion, whereas in Europe and Japan it is expected to reach $15 and $10 billion respectively by the year 2000 (1).

It is clear that the success of these products is mainly due to the scientific information, today available to most people. Thus the idea that some natural substances can play a significant role in disease prevention is generally accepted. In this contest, nutraceuticals are considered to provide a vehicle for a wide class of natural compounds. Not only could these decrease the incidence of the most important diseases in man, such as heart disease for instance by reducing cholesterol and blood pressure, but they could also inhibit several types of carcinogenesis. As regards the latter, it is important to emphasise that although over the last two decades attempts to treat the most important forms of cancer in man have been intensified, in some cases obtaining important results, most cancers are still difficult to cure. For this reason, prevention appears to be an essential strategy, especially as it also reduces social health care costs, an important item in most countries.

Today many molecules used in clinical practice for cancer therapy are natural products or their derivatives. Amongst these, plant-derived compounds play a dominant role in pharmaceutical therapy and in particular in antitumor drugs. The most important and innovative examples are Paclitaxel (Taxol^{R}), Vincristine (Oncovin^{R}), Podophyllotoxin and Camptothecin (Hycamtin^{R}), which are isolated from *Taxus* *brevifolia, Catharanthus roseus, Podofillum peltatum* and *Camtotheca acuminata* respectively (2).

Of course there are many other drugs derived from natural bioactive compounds often prepared using enzymes. In many of these cases, it is easy to appreciate that the high selectivity, and soft reaction conditions associated with enzymatic procedures, have made them very attractive for the preparation of complex bioactive and enantiopure compounds, which are often difficult to obtain with chemical methods.

Among the many natural bioactive compounds which can be used to produce healing formulations, glucosinolates (GLs) and their enzymatic degradation products (GLDPs) are strong candidates, due to their generally high bioactivity and the variety of derived-compounds that can be obtained. Thanks to these properties, there is a great potential for use of GLs in fine chemistry, food technology and medical applications.

Glucosinolates and their chemistry

Mustard relishes and various types of cruciferous vegetables containing GLs or GLDPs have for centuries been appreciated for their smell and flavour, e.g. mustard and. Nevertheless, GLs, and especially GLDPs, have mainly been considered as anti-nutritional compounds, in relation to the use of feed containing cruciferous products, e.g. rapeseed defatted meal. However, in the last decade, positive effects of GLs derivatives in preventing some diseases have also been observed in connection with the use of cruciferous-based food (3). Therefore, pure GLs could offer a useful resource when preparing high value nutraceutical formulations to prevent cancer in man.

GLs constitute a homogeneous family of more than 100 metabolites present mainly in the *Brassicaceae* family, with a different chemical conformation depending on the structure of side-chain, which may be aliphatic, aromatic or indolic. GLs are sugar anionic thioesters containing a β-thioglucoside-type bond, which can be quickly hydrolyzed by MYR-catalyzed reaction to give stoichiometric amount of D-glucose, hydrogen sulfate ion, generating a series of diverse aglucons such as isothiocyanates (ITCs), nitriles (NIs), thiocyanates, thiones (OZTs) etc.. This strictly depends on the chemical structure of the GLs side chains and the reaction conditions used. For instance, pH values close to neutral induce the production of ITCs, whereas acidic conditions induce the formation of ITCs and NIs together. The presence of a reducing agent (ferrous ion, cysteine) causes the formation of NIs mainly. In the case of ITCs with a hydroxy group in the β position with respect to the ITC function, a fast cyclization process occurs to give chiral OZTs.

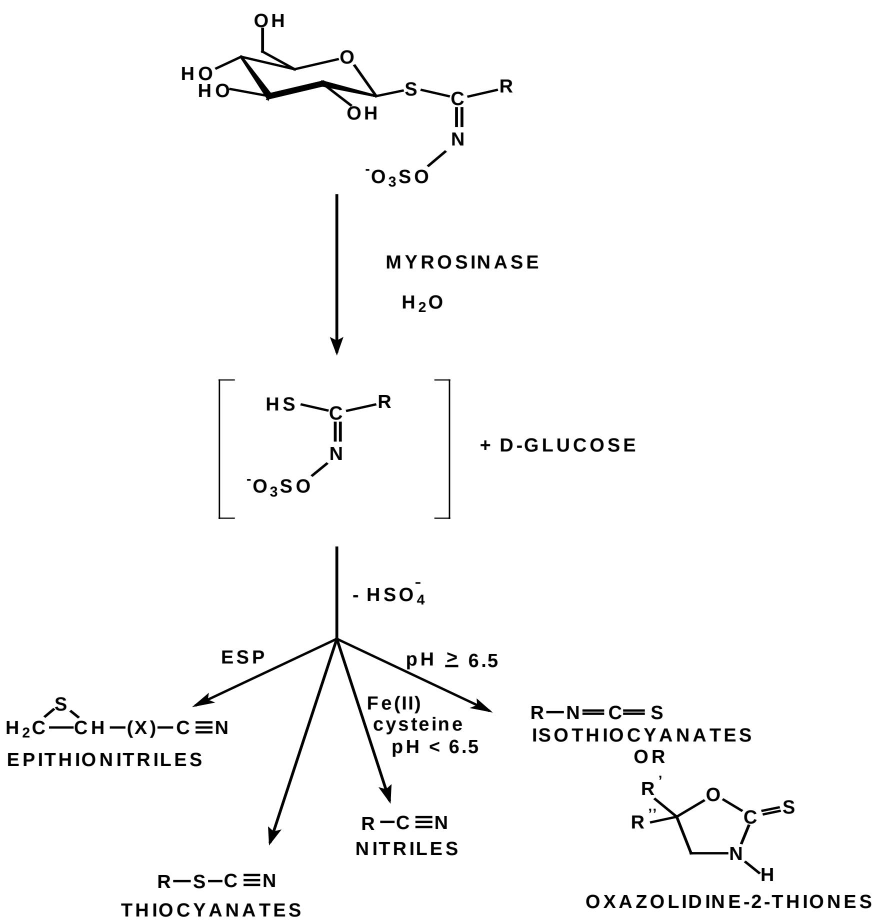

Fig.1 - Myrosinase-catalyzed hydrolysis of glucosinolates

MYRs, together with GLs, are present in all species of the *Brassicaceae* plant family and particularly abundant in the seeds where they are synthesised in the endoplasmatic reticulum. They are stable glycopolypeptides containing various disulfide and salt bridges, as well as a high percentage of carbohydrates, mainly hexoses (4).

Other interesting molecules can be obtained from GLs when the sulfate group is removed. GLs which do not have acylated thioglucose moieties can also be hydrolyzed by a special sulfatase (SUL) that we have isolated from *Helix pomatia* and immobilized on nylon 6.6 (5). The desulfo-GLs (DSGLs) produced in this reaction are potentially useful for different and innovative applications, owing to their chemical structure and physiological effects. DSGLs, in addition to their importance from the analytical point of view (6), can also be prepared as enantiomerically pure thioglucosidic compounds and considered as sophisticated
buildings blocks for synthesising new bioactive structures (5).

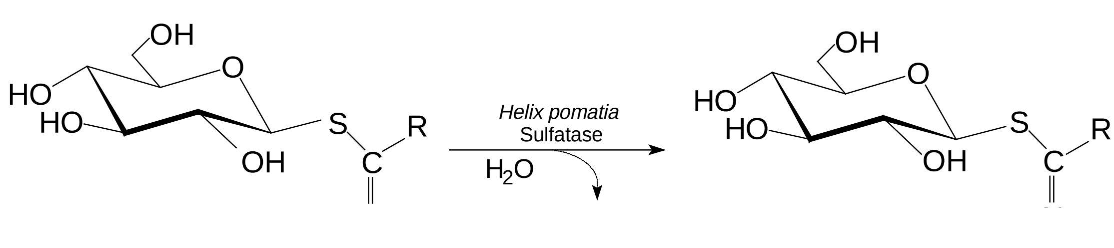

Fig.2 - Sulfate anion removal by sulfatase catalyzed hydrolysis of GLs.

Recent studies carried out in our laboratory revealed another important way to use DSGLs. Using a transgenic β-O-Glucosidase as catalyst, DSGLs are hydrolysed to produce the correspondent pure nitrile (7). The latter cannot be prepared in pure form using MYR because, in this case, varying amounts of isothiocyanates are even produced, depending on the hydrolyzed GL.

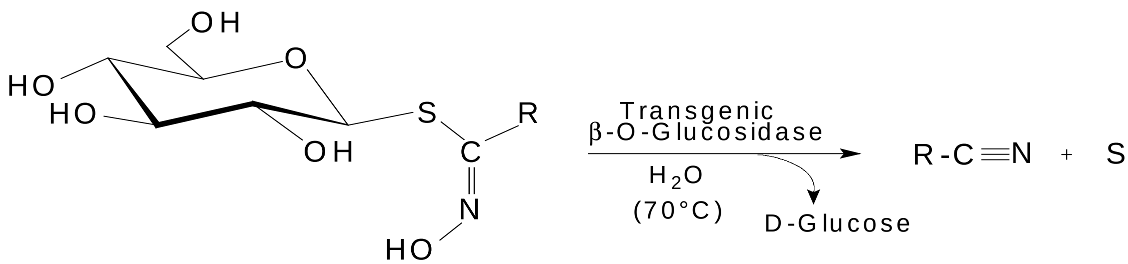

Fig. 3- Nitrile production by transgenic β-O-glucosidase catalyzed hydrolysis of DSGLs

Myrosinase and sulfatase technology

We applied different techniques to prepare GLDPs (8). In previous studies, we demonstrated that pure GLs can be isolated with a good yield at a laboratory level, starting from different kinds of ripe cruciferous seeds (9). Even MYR and SUL enzymes can be easily isolated in pure form from ripe *Sinapis alba* seeds and *Helix pomatia* snails respectively (6,10). We also found that MYR and SUL, isolated with our procedures, could be advantageously used in enzyme technology, almost like industrial catalysts, due to their special physicochemical properties.

We used these enzymes in free form in water solution and sometimes solubilized in reverse micelles, mostly when the reaction product showed low water solubility (11). MYR and SUL were used even in immobilized form on organic and inorganic supports, depending on the physicochemical characteristics of the starting GLs and mainly on the final GLDPs, especially their water solubility and inhibitory properties on the enzymes (12). In all cases, we established that MYR and SUL showed an extraordinary standing and operational stability, even at temperatures at which the majority of enzymes are deactivated. These findings emphasize the high potential of these enzymes for large-scale processes, although in this case GLs would have to be available in large amounts. Today, however, there are good technical and environmental reasons to suggest that these natural compounds will soon be available on the market in reasonable quantities as industrial co-products. Thus a sufficient availability of different kinds of GLs would make the GLs-MYR and the GLs-SUL systems efficient and environmentally-friendly ways to produce a wide assortment of GLDPs. These could be applied in food technology but also in medicinal chemistry for cancer prevention.

One sure approach to cancer prevention is diet optimisation, giving priority to the consumption of plant-based food. In fact several authors report that a diet rich in cruciferous vegetables (Brussels sprouts, cabbage, broccoli, radish, cauliflower, etc.) are important for reducing the risk of cancer, mostly colorectal cancer in man (13-17). The beneficial effects in cancer prevention of these vegetables appears to be strictly correlated to GLs content and especially to the GLDPs, which become available after *in vivo* metabolization of GLs by endogenous or commensal microflora enzymes with a MYR or MYR-like activity.

The case of glucoraphanin and sulforaphane

Recent results indicate the importance of some GL-derived isothiocyanates due to their cancer prevention activity; they are able to enhance the activity of phase II enzymes, considered to play an important role in the detoxification of xenobiotic compounds assimilated with diet (18). One of these is the metylsulfinyl butyl isothiocyanate commonly named sulforafane, which is currently produced by MYR-catalyzed hydrolysis of glucoraphanin (GRA); the most important GL contained in broccoli (*Brassica oleracea* cv. Italica).

In 1997, Talalay and coworkers demonstrated that a raw broccoli freeze-dried extract was a potent phase II enzyme inducer (18). After these important studies, this nutraceutical formulation was patented (US Patent n.5,411,986) and now is available on the nutraceutical market as efficient food integrator to prevent cancer. Nevertheless, since then, the validity of this product in cancer prevention has been the object of criticisms and is still the subject of debate.

A part from the clinical aspect, and at least from a biochemical point of view, it is important to establish whether GRA in broccoli is the only true effector controlling the enzyme systems mentioned. In principle, we need to know if there are contributions of other GLs, as in broccoli seeds and sprouts GRA is associated with 6 other GLs, which together represent 40-50% of the total GLs content (19). For instance, in our previous studies, carried out on proliferating human erythroleukemic K562 and other tumoral cells, using nine GLDPs produced by MYR-catalysed hydrolysis of pure GLs with *pre-mix* and *in situ* techniques, we also demonstrated a clear inhibition of proliferative growth also with sinigrin, glucotropaeolin, glucoerucin, glucocheirolin ($IC_{50} < 20$ μM) (20).

At this point it is important to clarify the real role of GRA in cancer prevention, by carrying out suitable *in vitro* and *in vivo* experiments using pure GRA. In fact, it is of fundamental importance to understand the active mechanism of sulforaphane on phase I and phase II enzyme systems. Without this knowledge there is the risk that, in certain circumstances, mainly with a high effector concentration, these two enzyme systems could generate dangerous reactive intermediates, which could attack cellular macromolecules, leading to toxicity and in some instances carcinogenesis. So at present the issue is still quite confusing, essentially due to the dual nature of phase I and phase II enzyme systems, as well as the huge number of compounds that can affect their activity.

In conclusion, it is reasonable to suggest that the original question concerning the activity in cancer prevention of GLs and their ITCs derivatives, as pure molecules in nutraceutical formulations, at present remains open with no convincing answer. On the other hand, the beneficial effects of GLDPs produced by eating cruciferous vegetables or freeze-dried tissues is quite well accepted, although there is hesitation regarding the possible inhibition or synergistic effect produced by molecules of different types contained in crucifers.

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