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Transport of resveratrol, a cancer chemopreventive agent, to cellular targets: plasmatic protein binding and cell uptake

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Abstract

Resveratrol produced by several plants, berries and fruits, including grapes, is one of the best known natural food microcomponents with potent chemopreventive properties towards the most severe contemporary human diseases: cardiovascular sickness, cancer and neurodegenerative pathologies. Demonstration of its mechanism of action also implies the elucidation of the steps of bioavailability and bioabsorption in cells and tissues. In order to estimate the relationships between the amounts of resveratrol taken up by food or drink intake, and the several possible benefits illustrated from in vitro/in vivo experiments and from epidemiological studies, it is essential to demonstrate step by step the route of resveratrol from plasma to the cell active site. In plasma, resveratrol was shown to interact with lipoproteins. This commentary also contains previously unpublished results about interactions between resveratrol and albumin and the enhancement of this binding in presence of fatty acids. We have previously described that resveratrol uptake by hepatic cells involves two processes – a passive one and a carrier-mediated one. Thanks to this last process, resveratrol, while tightly bound to blood proteins, could be largely delivered to body tissues. The intracellular proteic targets of resveratrol remain to be identified.

Keywords: Resveratrol; Albumin; Protein binding; Cell uptake; Fluorescence; Exclusion chromatography

1. Introduction

As a phytoalexin, resveratrol (3,5,4′-trihydroxystilbene) is a well-known grape and wine polyphenolic microcomponent, synthesized in grape skin in response to infection by *Bothrytis cinerea*, whose proliferation it blocks. Numerous studies have reported interesting properties of *trans*-resveratrol as a preventive agent against several important pathologies: vascular disease, cancer, viral infection and neurodegenerative processes (for reviews, see [1–4]). In addition, as recently reported, resveratrol may also increase yeast lifespan [5]. Moreover, several epidemiological studies (in particular [6]) revealed that resveratrol would be one of the main wine microcomponents that are beneficial for health in moderate wine consumption. On the other hand, the antiproliferative effect of resveratrol has been shown in vitro in several cell lines derived from tumors. For instance, we have described such effect of resveratrol in human hepatoblastoma-derived HepG2 cell line [7], and shown an apoptotic process in SW480 cancer colorectal cell line [8,9]. Although the biological positive effects of resveratrol are largely admitted, little is known about the transport and the distribution of resveratrol through the body. Due to its low water solubility [10], resveratrol must be bound to proteins and/or conjugated to remain at a high concentration in serum. Moreover, the efficiency of a therapeutic substance is related to its capacity (selectivity and affinity) to bind protein transporters [11]. This report shows unpublished data on the molecular interaction of resveratrol with albumin. Recently, we have shown the involvement of both passive diffusion and carrier-mediated processes in resveratrol uptake by hepatic cells [12]. We have also shown the consequence of resveratrol binding on its uptake. Albumin appears to be one of the plasmatic carriers transporting resveratrol in blood circulation in order to deliver the compound at the cell surface before cell membrane uptake and finally allowing its intracellular biological effect.

Abbreviations: BSA, bovine serum albumin

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2. Interaction of resveratrol with serum proteins

Dietary resveratrol is largely absorbed at the intestinal level as shown by studies on human intestinal Caco-2 cells [13] and on human healthy subjects [14]. It is further distributed to tissues and is retrieved mainly in liver and kidney, but also in other tissues as colon, lung, heart and brain [15,16]. To investigate the extent and the rate of resveratrol binding to plasma proteins, we have studied the time-course of the association of resveratrol with serum proteins by incubation of resveratrol (5 μM) with a standard cell culture medium containing 10% fetal calf serum. After incubation, unbound resveratrol was extracted with ethyl acetate and quantified by HPLC analysis. The rate of resveratrol binding is represented in Fig. 1. The proportion of unbound resveratrol decreased to 50% after 2 h and tended towards 0 after 24 h, meaning that when used at the initial concentration of 5 μM, all incubated resveratrol was bound to proteins after 24 h.

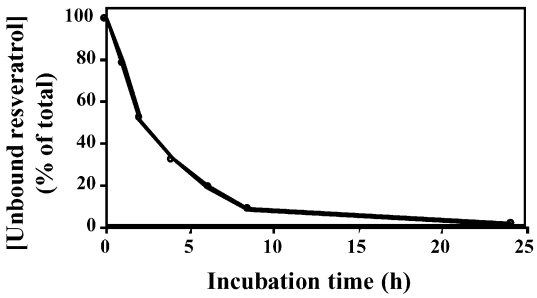

Fig. 1. Kinetics of resveratrol binding to serum proteins of culture medium. *Trans*-resveratrol (Sigma, initial concentration 5 μM) was incubated for different times at 37 °C (pH 8) with standard cell-free culture medium. Unbound ligand was extracted by ethyl acetate and then quantified by HPLC analysis. The analyses were performed on a reversed-phase Nucleosil C_{18} column (250 × 4.6 mm, 5 μm) from Touzart and Matignon. A Waters 625 LC system, including a gradient controller and a fluid handling unit with a Rheodyne 7125 injector, was used together with a Waters 486 Tunable absorbance detector and a SP4400 ChromJet integrator (Spectra-Physics). The UV-detector was set at 306 nm and stilbenes were eluted from the column with a gradient containing water and acetonitrile. Resveratrol concentrations in culture media were calculated by using a standard curve of resveratrol.

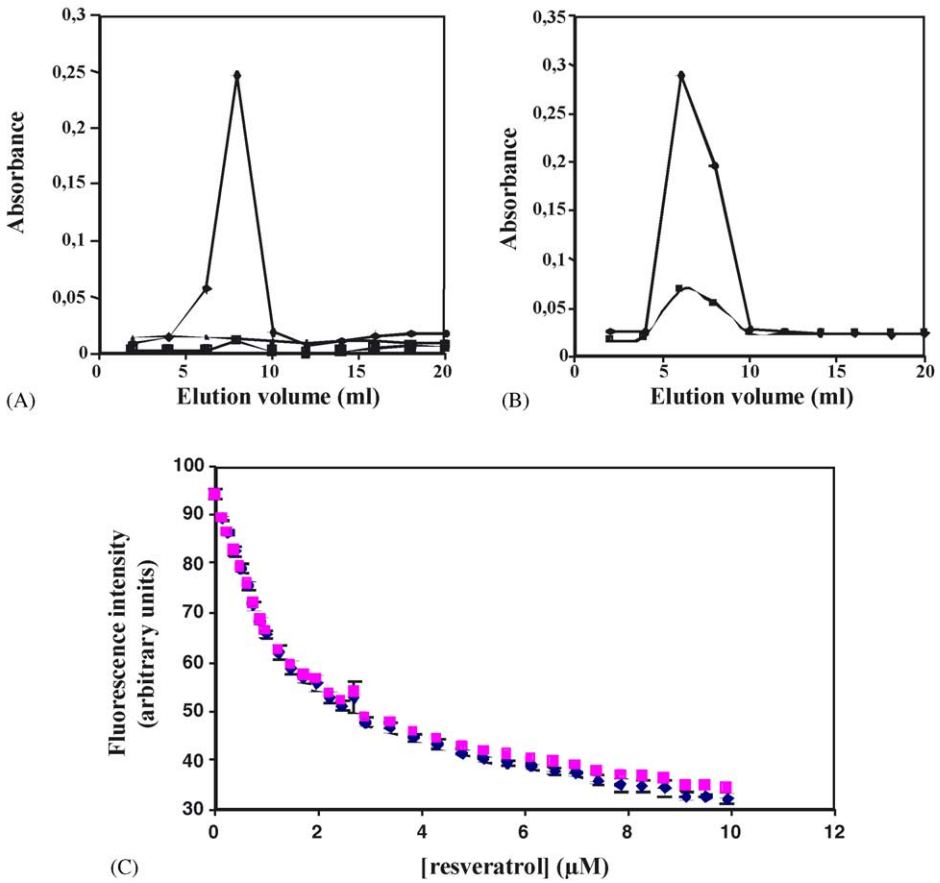

Fig. 2. Evidence for resveratrol binding to BSA. Panels A and B: Comparison of gel filtration assays on Sephadex G25 of BSA before and after interaction with resveratrol. Panel A: Elution curves of BSA (fraction V, Sigma) alone at 280 nm (◆) or 320 nm (■) and of resveratrol alone at 320 nm (▲). Panel B: Elution curves of BSA preincubated with resveratrol at 280 nm (◆) and at 320 nm (■). Panel C: Fluorescence measurements carried out with a Kontron SFM 25 spectrofluorimeter. Quenching of albumin fluorescence in presence of increasing concentrations of resveratrol (λ excitation: 295 nm; λ emission: 350 nm); (◆) fluorescence intensity (mean from three measures); (■) fluorescence intensity after correction of internal filter effect (IFE).

3. Interaction of resveratrol with albumin

Since albumin is well known to bind and carry out a large number of amphiphilic molecules, it is a good candidate as resveratrol plasmatic carrier. For example, it was demonstrated that quercetin, another polyphenolic compound, interacts with a high affinity with albumin in rat plasma [17] and with human serum albumin [18]. Interaction of resveratrol with BSA was also evidenced by an ultrafiltration method [19]. In order to study interaction between resveratrol and albumin, we have performed binding assays using BSA, with different crossed methods, including gel filtration chromatography and spectrofluorimetry. First, BSA alone and BSA pre-incubated with resveratrol were analyzed in the same conditions on a G25 Sephadex column (Fig. 2A and B). In the two cases, the protein fraction excluded from the gel was collected and spectrophotometric analysis was performed at 280 nm (wavelength characteristic of proteins) and at 320 nm (characteristic of *trans*-resveratrol). The absorbance at 320 nm increased in the experiment with BSA incubated with resveratrol, demonstrating the formation of resveratrol–albumin complexes. Second, we compared the fluorescent properties of resveratrol alone or in presence of BSA. Resveratrol fluorescence was measured at an excitation wavelength of 306 nm and at an emission wavelength of 400 nm. We observed an increase of the fluorescence intensity of resveratrol after its interaction with BSA (see Fig. 3) due to the formation of complexes modifying the microscopic environment of the fluorophore. An increase of fluorescence intensity was already described with quercetin and indicated that this ligand binds albumin at a site near tryptophan residue [18,20]. Finally, when fluorimetric measures were performed at a λ excitation of 295 nm and at a λ emission of 350 nm, we observed a quenching of the fluorescence of tryptophanyl residues of BSA after its binding to resveratrol. Fig. 2C shows the dependency of this quenching on ligand concentration. All these methods confirmed the interaction of resveratrol with albumin, and we continue our investigations to evaluate the resveratrol–BSA binding stoichiometry by the variation of fluorescence anisotropy and the number of moles of quercetin bound per mole of protein (BSA) by the Scatchard's procedure.

4. Enhancement of resveratrol binding to serum albumin in presence of fatty acids

The effect of fatty acids on the binding of resveratrol to BSA was determined by two methods: exclusion chromatography and spectrofluorimetry at weak concentrations of fatty acid-free albumin or albumin fraction V (Fig. 3). In the assays of exclusion chromatography performed at various initial concentrations of BSA

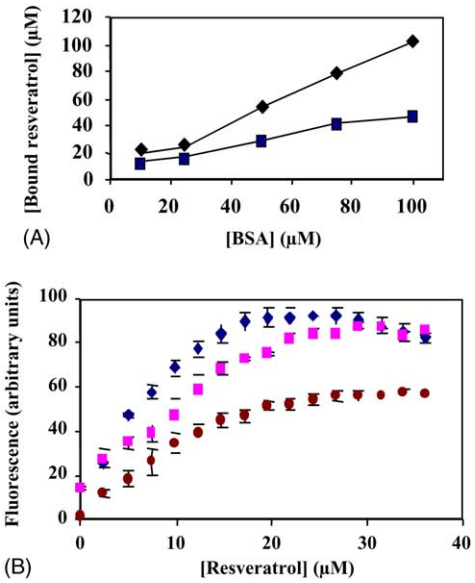

Fig. 3. Comparison of binding to fatty acid-free BSA and to fatty acid-containing BSA. Resveratrol (10^{-3} M) was incubated at room temperature (pH 7.4) for 18 h with various concentrations of fatty acid-free BSA from Sigma (■) or BSA fraction V (◆). Panel A: Bound resveratrol was separated on a Sephadex column. The incubated mixtures were filtered on a 7-cm column filled with Sephadex G25 fine and protected from light with aluminum foil. Elution was performed with 0.1 M phosphate buffer (pH 7.4) and monitored with absorbance detector (Isco) at 280 nm. Fractions containing free and bound resveratrol were collected separately. Bound resveratrol concentration was calculated from the absorbance values at 320 nm and at 280 nm to correct from protein absorption. Panel B: Analysis of resveratrol fluorescence exaltation. Fluorescence of resveratrol alone in buffer (●) or in presence of 5 µM BSA fraction V (◆) or in presence of 5 µM fatty acid free BSA (■) (λ excitation: 306 nm; λ emission: 400 nm).

(Fig. 3A), the concentrations of resveratrol bound to albumin fraction V were two times higher than the concentrations of resveratrol bound to fatty acid-free albumin. These results agree with those obtained by the spectrofluorimetric method showing that resveratrol fluorescence exaltation was higher when lipids are bound to albumin (Fig. 3B). Therefore, fatty acids have a positive effect on the binding of resveratrol to BSA. It is known that the shape of the protein is modified by the presence of fatty acids [21]. Fatty acids were usually used as vector because they present a high affinity for the liver and they have an efficient cellular uptake as a result of a specific interaction with the transmembrane transporter (liver plasma membrane–fatty acid-binding protein). As underlined by Bhattacharya et al. [22], it is difficult to predict the effects of fatty acids on drug binding because both cooperative and competitive interactions were reported. These authors showed that the drug-binding site named Sudlow's site I appeared to be a primary site for medium-chain fatty acids, while Sudlow's drug site II was likely to have a high affinity for long-chain fatty acids. Therefore, these two binding sites are not equally affected by plasmatic fatty acids that are essentially long chain fatty acids.

5. Resveratrol binding to other proteins

Proteins other than albumin may also be implicated in the high-affinity fixation of resveratrol. In general, red wine antioxidants bind to human lipoproteins and protect them from metal ion-dependent and -independent oxidation [23]. This protective effects of wine polyphenols might involve resveratrol, which might exert this effect by removing copper from LDL particles and arterial tissue and, thereby, delaying the consumption of flavonoids and endogenous antioxidants [10]. As the other red wine antioxidants, resveratrol can interact with lipoproteins [19,24]. In vitro assays showed that, on a protein basis, the concentrations of *trans*-resveratrol added to plasma increased with the order of their lipid content, i.e. HDL < LDL < VLDL, and that resveratrol is more associated with lipoproteins than with lipoprotein-free proteins [19]. This binding occurs also in vivo since the presence of dietary polyphenolic compounds was detected in human LDL isolated from blood samples of healthy volunteers [25]. Other polyphenols were reported to bind plasma proteins. For example, catechin is bound to human apo A-I and rat transferrin [26], genistein is bound to HDL and LDL [27]. Actually, we are studying if resveratrol can bind to other proteins, such as alpha-fetoprotein, which is a binding protein for many estrogenic compounds as xenoestrogens and phytoestrogens.

6. Trapping of resveratrol and cellular uptake

Using hepatoblastoma-derived cells (HepG2), we have studied the characteristics and the kinetics of labeled resveratrol transport. We have shown that resveratrol uptake involves both passive diffusion and a carrier-mediated process [12]. We have also shown that the rate of the passive transport of resveratrol was two-fold lower in serum-containing cell culture medium, compared to that obtained in serum-free medium. Moreover, an increasing lowering of resveratrol uptake was observed by addition of increasing concentrations of BSA to a serum-free medium. These results underline the importance of resveratrol binding to serum proteins and especially to albumin. The involvement of a carrier-mediated transport could allow a good uptake of resveratrol, at weak concentrations, despite of its trapping by serum proteins. It may be hypothesized that resveratrol–albumin complexes could be retained by albumin membrane receptors, and that resveratrol would then be delivered to the cell membrane, as described for fatty acid transport [28]. This hypothesis is very important when the biochemical data obtained on molecular and cellular models have to be replaced into an in vivo context. Indeed, the affinity and the binding of resveratrol to albumin can suggest that albumin could be a natural polyphenol reservoir in an in vivo context, where it might play a

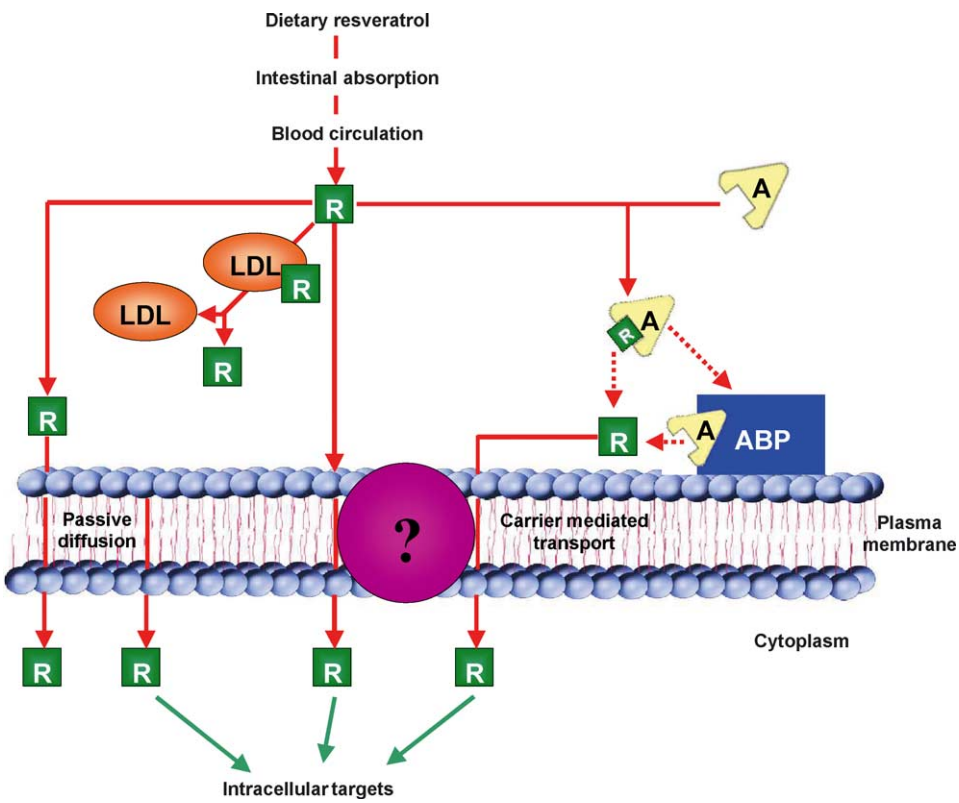

Fig. 4. Scheme of the hypothetical routes in resveratrol transport from blood circulation to intracellular targets. R: resveratrol; A: albumin; ABP: albumin-binding protein.

pivotal role in the distribution and bioavailability of circulating resveratrol.

7. Resveratrol binding to intracellular receptors

Little is known about the intracellular targets of resveratrol. It was shown to be a competitive antagonist of dioxin binding to aryl hydrocarbon receptor (AhR). Its promotes the AhR translocation to the nucleus [29]. Phyoestrogens, such as genistein and resveratrol, show some structural resemblance with human estrogens and are able to bind to estrogen receptors (ERs). Several authors have reported the interaction of resveratrol with estrogen receptors. Resveratrol binds to ER-alpha and ER-beta with comparable affinity but with a 7000-fold lower affinity than estradiol [30]. Molecular dynamics studies have shown that the binding of resveratrol to ER-alpha is stereoselective, with a weaker binding of the *cis* form compared to the *trans*-isomer [31].

8. Conclusion

The possible routes of resveratrol transport from plasma to intracellular targets are schematically represented in Fig. 4. This scheme concerns only the transport of the unconjugated form of resveratrol. It is known that resveratrol is conjugated in intestine as glucuronide and sulfate derivatives, but aglycone is also bioavailable [16,32]. Therefore, we will follow our investigations about the transport of resveratrol, of its metabolites, as well as that of synthetic analogues with interesting biological effects.

Acknowledgments

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