[S-3]

A selective measurement of antioxidant capacity in both the aqueous and lipid compartments of plasma

Giancarlo Aldini[1] and Kyung-Jin Yeum[2]

[1]*Istituto Chimico Farmaceutico Tossicologico, University of Milan, Viale Abruzzi 42, 20131 Milan, Italy.*
[2]*Jean Mayer USDA-Human Nutrition Research Center on Aging, Tufts University 711 Washington St, Boston, MA 02111, USA.*

The measurement of the total antioxidant capacity (TAC) of human plasma has been applied in the nutrition science in several ways. In particular, it has been used to evaluate the antioxidant contributions of dietary components and to study the bioavailability of dietary antioxidant nutrients.

Several methods have been recently proposed for the measurement of the TAC in human plasma, most of them based on the measurement of the ability of plasma to withstand the oxidative damage induced by aqueous radical initiator such as AAPH or transition metal ions. However, as the oxidation process is induced primarily by hydrophilic radicals and measures the aqueous plasma compartment oxidizability, these methods are unable to determine the antioxidant capacity of the lipid compartment. It is not surprising that most of the methods used to measure the total antioxidant capacity of plasma such as TRAP (Total Radical Trapping Antioxidant Parameter), ORAC (Oxygen Radical Absorbance Capacity), and FRAP (Ferric Reducing Ability of Plasma) failed to show any direct correlation between fat-soluble antioxidant nutrient levels and antioxidant capacity in circulation following a diet enriched with carotenoids[1-3]. These results can be explained by considering that the plasma carotenoids, being deeply embedded in the lipoprotein core, are not available for reacting with the aqueous radical species or ferric complexes.

The selective measurement of lipid compartment oxidizability could be relevant not only to study the effect of a diet supplemented with fat-soluble antioxidants, but also to investigate the lipoprotein oxidation in whole plasma and the activity and mechanism of both fat- and water- soluble antioxidants. Therefore, we firstly focused our interest on developing a method capable of measuring and distinguishing the aqueous and lipid plasma compartment oxidizability. In particular, to monitor the lipid compartment plasma oxidation we used the azo compound, 2,2-azobis(4-methoxy-2, 4-dimethylvaleronitrile) (MeO-AMVN) as a lipid soluble radical initiator, and (4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (C11-BODIPY581/591) as a lipophilic fluorescence probe. At the same time, the aqueous plasma oxidative reaction was monitored using AAPH as the

aqueous peroxyl radical generator and 2,7-dichlorodihydrofluorescein (DCFH) as the marker of the oxidative reaction.

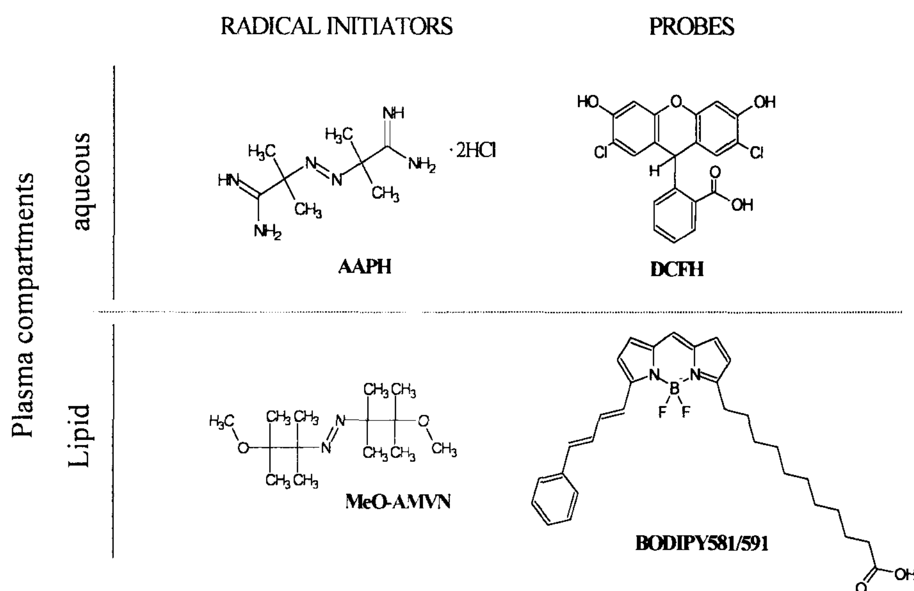

Figure 1: Structure of hydrophilic and lipophilic azo-compounds and probes for measuring aqueous and lipid compartments lipid oxidizability.

The selective distribution of the two radical initiators was confirmed by measuring the rate of consumption of hydrophilic and lipophilic endogenous antioxidants in plasma. In the presence of AAPH, hydrophilic radical initiator, we observed the following order of disappearance: ascorbic acid > -tocopherol > uric acid and -carotene. This indicates a gradient of hydrophilic peroxyl radicals from the aqueous to the lipid compartment of plasma. When MeO-AMVN, lipophilic radical initiator, was used as radical inducer, the order was partially reversed with -tocopherol > ascorbic acid > -carotene > uric acid. These results confirm the diffusion and activation of MeO-AMVN into the core of lipoproteins and also suggest that there is a great deal of interaction between -tocopherol and ascorbic acid, regardless of which initiating species is used. DCFH was used in the presence of AAPH to measure aqueous plasma oxidation. The selectivity of the method was confirmed inasmuch as DCFH oxidation only started after uric acid, the main hydrophilic plasma antioxidant, was consumed. In addition, when MeO-AMVN was used as the radical inducer, DCFH oxidation was significantly delayed, indicating its main localization in the aqueous domain.

To study the lipid oxidation process induced by MeO-AMVN, we used BODIPY581/591 as a lipophilic fluorescence probe for the following reasons: (a) it is characterized by a high fluorescence quantum yield limited to the lipid phase, (b) it is stable for several hours in biological fluids at 37°C,

(c) it absorbs/emits in the visible region, (d) it was found to be a sensitive and selective indicator of lipid plasma oxidation, (e) the initial peroxidation rate is similar to that observed for arachidonic acid. By adding MeO-AMVN, we observed the oxidative reaction whose rate constant significantly increased after the depletion of fat-soluble antioxidants such as -tocopherol and -carotene, while it was not apparently related to the water-soluble antioxidants levels. When hydrophilic radical initiator, AAPH, was used as the radical initiator, BODIPY581/591 oxidation was significantly delayed suggesting its localization in the lipid phase of plasma, and inaccessibility to the water-soluble peroxyl radicals generated from AAPH.

The measurement of lipid plasma oxidation was validated by adding two fat-soluble antioxidants, -tocopherol and -carotene, to plasma samples. As previously reported [4], pre-incubation of plasma after adding the fat-soluble antioxidants improves the enrichment of the plasma lipid compartments with the fat-soluble antioxidants. The protective effect against oxidation in the lipid compartment was found to be dependent on the duration of the pre-incubation period, suggesting a slow incorporation of -tocopherol and -carotene into the lipid compartment of plasma.

We have applied our method to a human study, to assess whether dietary carotenoids can modify human plasma lipid oxidizability. Volunteers were placed on a high fruit and vegetable diet for 8 weeks to increase their plasma carotenoids, and were then depleted of carotenoids by placing them on a low fruit and vegetable diet for 4 weeks. Under these circumstances, there is an inverse linear relationship between the lipid oxidizability of plasma and the content of plasma carotenoids [5].

We then studied the antioxidant mechanism of (-)-Epigallocatechin-(3)-gallate (EGCG), the main polyphenolic component of green tea, in the human plasma [6,7].

When human plasma is exposed to the hydrophilic radical initiator, AAPH, the aqueous compartment oxidation (monitored by DCFH oxidation) is dose-dependently inhibited by EGCG (IC_{50}= 0.72 μM), without sparing the hydrophilic antioxidants, ascorbic acid and uric acid. In contrast, the lipophilic antioxidants (-tocopherol and carotenoids) are significantly spared by 1-5 μM EGCG. When radical damage was selectively induced in the lipid compartment of plasma using MeO-AMVN as the lipophilic radical inducer, EGCG was found to spare -tocopherol, but not the carotenoids. In addition, EGCG inhibited lipid compartment oxidation, monitored by BODIPY581/591 as a selective lipid probe, but with a potency lower than that found in the aqueous compartment (IC_{50}= 4.37 μM). The results indicate that EGCG at concentrations found in human plasma after acute or chronic green tea ingestion is mainly localized in the aqueous compartment, where it is effective in quenching aqueous radical species, thus limiting their diffusion into the lipid compartment and preventing lipid-soluble antioxidant depletion. EGCG is also able to interact with -tocopherol at the aqueous/lipid interface where recycling of -tocopherol occurs through an H-transfer mechanism, as confirmed by ESR experiments, affording an additional protective mechanism to the lipid compartment of plasma.

References

1) Pellegrini N, Riso P, Porrini M. Tomato consumption does not affect the total antioxidant capacity of plasma. *Nutrition.* 16:268-271; 2000.

2) Castenmiller JJM, Lauridsen ST, Dragsted LO, van het Hof KH, Linssen JPH, West CE. -Carotene does not change markers of enzymatic and nonenzymatic antioxidant activity in human blood. *J Nutr.* 129:2162-2169; 1999.

3) Cao G, Booth SL, Sadowski JA, Prior RL. Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. *Am. J. Clin. Nutr.* 68:1081-1087; 1998.

4) Aldini G, Yeum K-J, Russell R.M.; Krinsky NI. A method to measure the oxidizability of both the aqueous and lipid compartments of plasma. *Free Radic. Biol. Med.* 31:1043-1050; 2001.

5) Krinsky NI, Aldini G, Yeum K-J, Russell RM Dietary carotenoids modify human plasma lipid oxidizability *Free Radic. Biol. Med.* 33: 657 Suppl. 1 2002

6) Aldini G, Yeum K-J, Krinsky NI, Russell, R.M. (-)-Epigallocatechin-(3)-gallate spares plasma lipid-soluble antioxidants, recycles vitamin E and prevents oxidative damage of both aqueous and lipid compartments in plasma. *FASEB J* 16 (4): A243-A243 Part 1 MAR 20 2002.

7) Yeum K-J, Aldini G, Krinsky NI, Russell, R.M. Dietary modification of antioxidant capacity in the lipid compartment of serum *FASEB J* 16 (4): A603-A603 Part 1 MAR 20 2002