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Review

Preanalytic of total antioxidant capacity assays performed in serum, plasma, urine and saliva

Cristiano Ialongo *

Department of Physiology and Pharmacology "Vittorio Erspamer", Sapienza University of Rome, Rome, Italy
Department of Laboratory Medicine, Tor Vergata University of Rome, Rome, Italy

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ABSTRACT

The investigation of oxidative stress (OS), its mechanisms and connections with human diseases, is a topic of interest with more than 36,000 PubMed citations to date. The OS can be approached either from the perspective of pro-oxidation, either of anti-oxidation, and both can be investigated considering individual chemical constituents or their pooled effect. Actually, as it is for any laboratory assay, whatever source of variability introduces a bias potentially undermining the test results regardless of its application. In this regard, the effect of sample collection, handling and storage – that collectively constitute the preanalytical phase - on the likeliness of the measured value represent a major challenge for any researcher. In this review, we will deal with methods devised to assess the so-called total antioxidant capacity (TAC), which represents the sinking capability expressed toward a given load of pro-oxidant species. Thus, it will be presented the information available to date on the preanalytical phase of TAS assessment, focusing on the issues that strictly concern the preservation of antioxidants within the specimen. Obviously, preanalytic should represent a first concern in any laboratory research, to which this work could contribute giving practical indications and raising the researchers' awareness about the issue.

Contents

1. Introduction

The investigation of oxidative stress (OS), its mechanisms and connections with human diseases, is a topic of interest with more than 36,000 PubMed citations to date. Literally, the OS represents "the disbalance in pro-oxidant/anti-oxidant equilibrium in favor of the pro-oxidant", that can arise either from an increase of pro-oxidants, either from a decrease of anti-oxidants (AOs) [1]. Indeed, living organisms rely on the energy transfer in which pro-oxidants and oxidation play a physiological role, so that the oxidative stress should be rather regarded as a steady-state biochemical process undergoing a tight biological

* Corresponding author at: Viale Oxford 81, 00135 Rome (RM), Italy.
E-mail address: cristiano.ialongo@gmail.com.

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control [2]. Thereby, it should be not surprising if the OS participates to almost all pathological processes and aging, being as such a major topic of clinical epidemiological studies [3]. The OS is also an effective model of the environment-organism interaction, either under the point of view of prevention, either by the perspective of a deeper interaction with the disease's physiology than the pro-oxidant/anti-oxidant equilibrium aforementioned [4,5].

The AOs can be divided into enzymatic and non-enzymatic systems, which in turn represent prevalently cellular or non-cellular mechanisms respectively [6,7]. To the first kind belong the superoxide dismutases (SOD), the catalases, the peroxiredoxins (Prx), the thioredoxin reductase (TR), the reduced glutathione peroxidase (GPx) and the oxidized glutathione reductase (GR). Conversely, to the second kind belong the compounds of 5 major classes:

a. Endogenous low-molecular-weight AOs: free reduced glutathione (GSH), uric acid (UA), lipoic acid (ALA) coenzime Q10 (CoQ10), bilirubin, methionine and cysteine
b. Exogenous low-molecular-weight AOs: ascorbic acid (vitamin C), the tocopherols (vitamin E) and the carotenoids (beta-carotene and vitamin A)
c. Proteins: albumin, cysteine-rich proteins
d. Polipeptidic AOs: thioredoxins, glutaredoxins and sulfiredoxins
e. Metal-binding proteins: ceruloplasmin and metallothioneins

Obviously, exogenous and endogenous AOs cooperate to counteract the pro-oxidants, giving rise to a network of interactions aimed to reduce the burden of oxidation (e.g. urates ➔ ascorbic acid ➔ tocopherols) [8,9].

Thus, the OS can be approached either by the perspective of pro-oxidation, either from anti-oxidation, and both can be investigated considering individual chemical constituents or their pooled effect. In this review, we will deal with methods devised to assess the so-called total antioxidant capacity (TAC), which represents the sinking capability expressed toward a given load of pro-oxidant species [10]. Such methods are of particular interest when applied to biological matrices, in that the overall effect measured taking collectively the AOs is suitable to show the potential response toward OS [10,11]. Moreover, the TAC returns a numerical measure suitable to compare the effectiveness or estimate the potential contribution of endogenous and dietary anti-oxidants [12,13]. Actually, as it is for any laboratory assay, whatever source of variability introduces a bias potentially undermining the test results regardless of its application [14]. Of course the analytical error can be easily assessed by means of quality control samples, which are integral part of any laboratory assay. Conversely, the effect of sample collection, handling and storage on the likeliness of the measured value can be controlled very hardly afterwards [15]. Hence, the overall test reliability also bases on the awareness of criticalities arising along the sample supply chain (namely the preanalytical phase), that can be controlled through the knowledge and effective compliance with procedures. Thus, primary aim of this manuscript is to review the information available regarding the preanalytical phase of TAS assessment, focusing on the issues that strictly concern the preservation of AOs within the specimen. Consequently, we also aim to provide evidence on how to properly manage samples in advance of the analytical assessment of TAC.

2. Chemical assays of TAC

The next discussion will briefly illustrate the basic concepts of chemical assays necessary to appreciate the preanalytical issues of TAC assessment discussed further on in this manuscript (to get a deeper insight of the topic the reader can refer to the much more extensive and comprehensive reviews already issued) [6,7,16–20]. Thus, since the TAC represents the moles of oxidants that are scavenged by a solution of AOs, the various proposed tests measure the rate of consumption of AOs, that turns out to be proportional to their concentration [10,21]. The methods devised to quantitate TAC through a molecular probe can be distinguished on the way the probe acts with respect to the free-radical chemistry [6]. A general model of autoxidation can be described as a four-step process [6]:

- Initiation (where S is a generic substrate):
  - o $R\bullet + O_2 \rightarrow ROO\bullet$
  - o $ROO\bullet + SH \rightarrow ROOH + S\bullet$
- Chain propagation
  - o $S\bullet + O_2 \rightarrow SOO\bullet$
  - o $SOO\bullet + SH \rightarrow SOOH + S\bullet$
- Inhibition/chain-breaking (where A is a generic AO)
  - o $SOO\bullet + AH \rightarrow SOOH + A\bullet$
- Termination
  - o $A\bullet + (n\text{-}1)SOO\bullet \rightarrow$ non-radical products
  - o $SOO\bullet + SOO\bullet \rightarrow$ non-radical products

Thereby there are two distinct possible reactions and measurements thereof:

- o The hydrogen atom transfer (HAT), in which it is measured the quenching capacity of free radicals by hydrogen donation (i.e. $R\bullet + AH \rightarrow RH + A\bullet$)
- o The single electron transfer (SET), in which it is measured the reducing capacity toward any molecule by electron donation (i.e. $R\bullet + AH \rightarrow R^- + AH\bullet^+$).

Thus, it is possible to classify the major analytical methods basing on the classification above:

- o HAT-based methods:
  - o total radical trapping antioxidant parameter (TRAP) [22]
  - o oxygen radical absorbance capacity (ORAC) [23]
  - o total oxyradical scavenging capacity (TOSC) [24]
  - o crocin bleaching assay [25].
- o SET-based methods:
  - o ferric ion reducing antioxidant power (FRAP) [26]
  - o copper reduction assay (CUPRAC) [27]
  - o mixed (primary SET and secondary HAT) methods:
  - o trolox equivalence antioxidant capacity (TEAC) [28]
  - o 2,2-Diphenyl-1-picrylhydrazyl assay (DPPH) [29].

In all such cases the probe can be detected by means of a spectrophotometer, a luminometer, a fluorimeter or a gas-chromatography apparatus [6].

The HAT-based methods are all competitive assays, since the endogenous AOs are measured through their capacity of preventing the degradation or inducing the restoration of the chemical probe acting as oxidation substrate [30]. In the TRAP and ORAC assays, the probe is a molecule that preserves its natural fluoresce (B- or R-phycoerythrin or fluorescein) as long as it is protected from oxidation by the AOs in the sample [23,31,32]. Similarly, in the crocin assay, the monitored reaction is the bleaching of the hydrophilic carotenoid derivative, that has a strong golden-yellow hue when untainted (responsible of the saffron stain of foods and textiles) [33]. Lastly, in the TOSC it is monitored the production of ethylene due to the α-keto-γ-methiolbutyric acid (KMBA) oxidation, that can be detected chromatographically

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