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Amino acids and plasma antioxidant capacity

Short Communication

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Summary. Amino acids antioxidant capacity has been investigated and compared with the chain-breaking antioxidant activity of known compounds as ascorbic acid and Trolox. Basic and acidic amino acids and most of neutral ones showed no antioxidant capacity. On the contrary, tryptophan, tyrosine, cysteine and homocysteine showed antioxidant ability at concentrations which are within the usually reported physiological ranges.

These findings are discussed in connection with the antioxidant capacity ascribed to plasma proteins, as human serum albumin.

Keywords: Amino acids – Antioxidant capacity – Oxidative stress – Plasma proteins – Human serum albumin

Introduction

Proteins are a potential source of antioxidant capacity in human serum, accounting for 10–50% of total TRAP values (Wayner et al., 1987); Cao et al. (1993) claimed that bovine albumin per unit weight has a lower peroxyl absorbing capacity than the other plasma antioxidants; however, the serum protein fraction represents the major contributor to the oxygen-radical absorbing capacity (ORAC) value found in the whole serum.

Mostly, plasma proteins are considered preventive antioxidants, acting by sequestering and/or otherwise inactivating transition metal catalysts (e.g., transferrin, ceruloplasmin). Recently, plasma proteins were discovered to be chain-breaking antioxidants, too (Wayner et al., 1987), their activity appearing to be mainly due to the presence of sulphydryl groups. However, other protein radical traps are suggested to account for total proteins contribution to protection against oxidative damage. The amino acid most likely to exhibit antioxidant activity is tyrosine, which contains (like vitamin E) a phenolic hydroxyl group. Monomeric tyrosine did not present observable antioxidant behaviour in aqueous solution in TRAP assay (Wayner et al., 1987). Similarly,

monomeric tryptophan and histidine showed no chain-breaking antioxidant activity (see again Wayner et al., 1987). Of course, in the proteins some of these residues may be present in much less polar, non-aqueous environments and such residues should be capable of trapping radicals. Whether they actually would do so would depend on whether the radicals could penetrate the protein to reach these potential radical-trapping sites (Wayner et al., 1987).

Therefore to better elucidate all of the radical-trapping reactions of proteins, we have tested the main amino acids usually found in proteins and in plasma in a free form by a new method for measuring antioxidant capacity (Rice-Evans and Miller, 1994). The findings are discussed considering the antioxidant capacity showed by plasma proteins, as human serum albumin.

Materials and methods

Trolox ((±)-6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid) and hydrogen peroxide, 30 wt%, were purchased from Aldrich; 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS) from Fluka; myoglobin from horse heart (95–100%), human serum albumin (HSA), L-serine, L-proline, cis-4-hydroxy-L-proline, L-phenylalanine, L-methionine, L-cysteine, DL-homocysteine, L-homocysteine thiolactone, L-cystine, L-homocystine, L-leucine, L-isoleucine, L-aspartic acid, L-glutamic acid, L-lisine, L-histidine, L-arginine, L-tryptophan, L-tyrosine and diethylenetriaminepentaacetic acid (DTPA) from Sigma; L-(+)-ascorbic acid from Merck.

All other chemicals used were of reagent or spectrophotometric grade.

Highly purified water (resistivity = 18 Mohm·cm) obtained through a Milli-Q water purification system (Millipore) was used for all solutions. Amino acids were dissolved daily and 10 mM solutions were prepared in phosphate buffered saline (PBS) (20 mM, pH 7.4), containing 0.5 mM DTPA, and kept protected from light in a sealed tube on ice, until used. DTPA was added to chelate adventitious redox-active metal ions able to catalyze amino acids oxidation.

HSA concentration is expressed in molar units, applying a M_r of 65,000 (He and Carter, 1992).

Absorbance was measured with a Hewlett-Packard 8450 A UV/Vis spectrophotometer equipped with a cuvette stirring apparatus and a constant temperature cell holder.

Measurements of pH were made with a PHM84 Research pHmeter (Radiometer); the electrode response was corrected for temperature.

Antioxidant capacity

Antioxidant capacity was determined according to Rice-Evans and Miller (1994) with a novel method based on the inhibition by antioxidants of the absorbance of the radical cation $ABTS^{\cdot+}$ formed by the interaction of ABTS (150 μM) with the ferrylmyoglobin radical species, generated by the activation of metmyoglobin (2.5 μM) with H_2O_2 (75 μM). The manual procedure was used with only minor modifications, i.e.: temperature at 37°C instead of 30°C to amplify the phenomenon and each sample assayed alone to carefully control timing and temperature. The reaction was started directly in cuvette with H_2O_2 after 1 min equilibration of all reagents (temperature control with a thermocouple probe, model 1408 K thermometer, Digitron Instrumentation Ltd.) and followed under continuous stirring, monitoring the absorbance at 734 nm for 7 min. Absorbance reading at 7 min is used to calculated the antioxidant capacity as the percentage inhibition of the reaction, i.e. the blank (with buffer alone) absorbance minus the text (with amino acid or other antioxidants) absorbance, divided by the blank absorbance (expressed as a percentage).

This value defines the response of the system and is proportional to the antioxidant capacity of the sample. 0.5–2.0 mM Trolox, a water-soluble analog of vitamin E, was also assayed in all experiments to control the system.

Results and discussion

In an effort to determine the antioxidant capacity of amino acid residues, almost all physiological amino acids and human serum albumin were assayed as described under "Materials and Methods".

When 10μM Trolox or vitamin C was added to the reaction mixture, the $ABTS^{\cdot+}$ absorption curve exhibited a lag phase followed by a fast increase of absorbance similar to that in the buffer blank; this suggests complete protection of $ABTS^{\cdot+}$ by these nonprotein-antioxidants. On the contrary, the addition of the same concentration of human serum albumin resulted in a minor increase of absorbance instead of producing a lag phase in the absorption curve, suggesting that it provides only partial protection for $ABTS^{\cdot+}$, as for peroxyl radicals (Cao et al., 1993) (Fig. 1). It is worth noting that HSA does not provoke a clear lag phase neither at very high concentrations (100μM).

No antioxidant activity was found when 0.5 mM solutions of arginine, hystidine, lysine, aspartic acid and glutamic acid, methionine, proline, hydroxyproline, phenylalanine, leucine, isoleucine and serine were tested. So a great lot of plasma amino acids (basic and acidic amino acids and most of monoamino, monocarboxylic ones) does not appear to contribute to the total

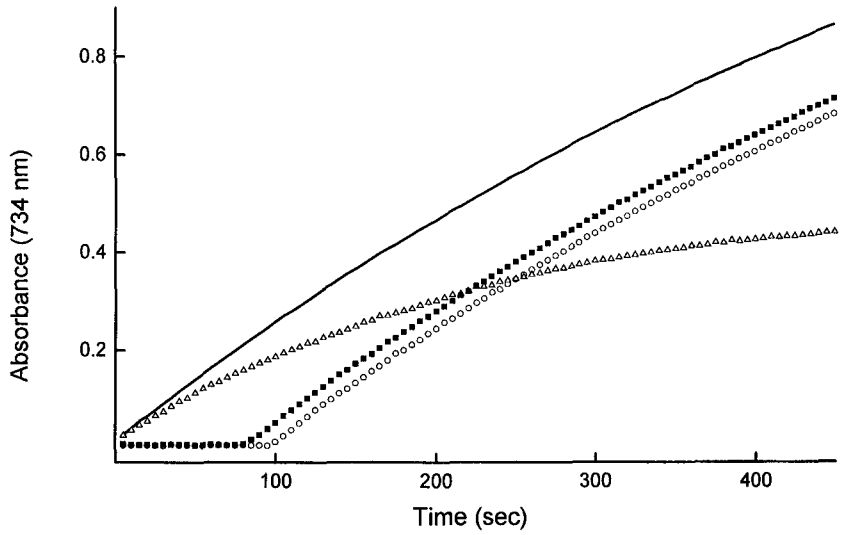

Fig. 1. Different pattern of inhibition of $ABTS^{\cdot+}$ absorbance by protein- and nonprotein-antioxidants. The antioxidant capacity of Trolox (10μM) (○), ascorbic acid (■) (10μM) and human serum albumin (10μM) (△) was assayed as described in "Materials and methods". The buffer blank is indicated as a continuous line and represents the increase of $ABTS^{\cdot+}$ absorbance in the absence of inhibitors. Data are representative of a typical result obtained in three separate experiments

plasma antioxidant activity, at least as assessed by the method described above.

On the contrary, tryptophan, tyrosine, cysteine and homocysteine present a good antioxidant activity. Besides, cysteine and homocysteine show antioxidant ability at very low concentrations (Fig. 2). Differently from human serum albumin, sulphur-amino acids show an inhibition pattern quite similar to that caused by nonprotein-antioxidants, i.e. a lag phase in the $ABTS^{\cdot+}$ absorption curve followed by a strong increase of absorbance. On the other hand, Romero et al. (1992) have demonstrated that thiols interact with myoglobin *via* two processes and that the oxidation of cysteine coupled with the reduction of the ferrylmyoglobin proceeds with formation of thiyl radicals by electron transfer from the thiol to the high oxidation state heme iron. Homocysteine thiolactone showed a very low antioxidant capacity only at high concentrations (0.25–0.50 mM); cystine and homocystine, too, showed antioxidant capacity, even if at concentrations much higher than the respective reduced compound (0.25–0.5 mM), without lag phase in the inhibition patterns. At the present, it is not clear the mechanism underlying these results, however the findings strengthen that sulphydryl groups represent a large expendable source of antioxidants in plasma (Wayner et al., 1987).

Tryptophan and tyrosine showed an inhibition pattern similar to that found in the presence of human serum albumin, i.e. a strong decrease of the absorbance of the radical cation $ABTS^{\cdot+}$; besides they do not have a clear lag at high concentrations (Fig. 2). Tyrosine and tryptophan were also found to be quite equivalent as antioxidant capacity extent at concentrations which are

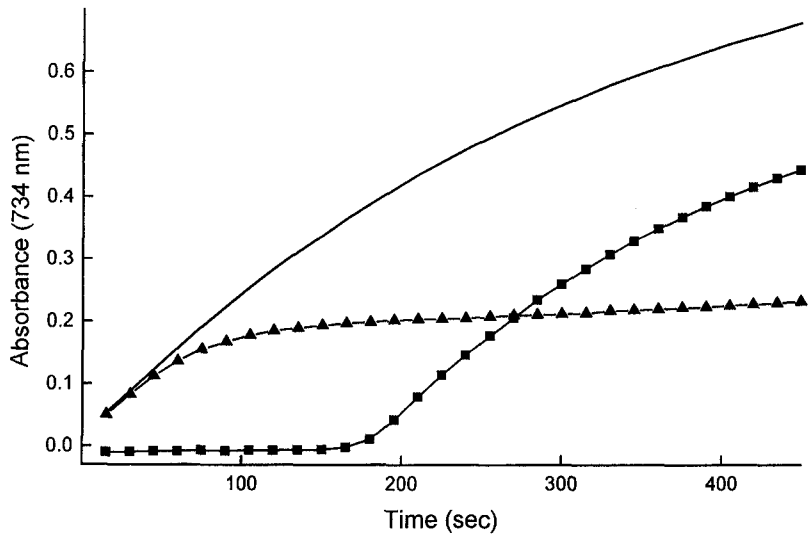

Fig. 2. Amino acids antioxidant capacity. The antioxidant capacity of tryptophan (16 μM) (▲) and cysteine (16 μM) (■) was assayed as described in "Materials and methods". DTPA (final concentration 25 μM) was added to prevent amino acids oxidation before addition to the system. The buffer blank is indicated as a continuous line and represents the increase of $ABTS^{\cdot+}$ absorbance in the absence of inhibitors. Data are representative of a typical result obtained in three separate experiments

within the usually reported physiological range (Roth et al., 1985). This result is in keeping with previous suppositions of radical-trapping reactions of the proteins not ascribed to sulphydryl groups and, at the time of writing, this is the first demonstration of the involvement of these amino acid residues in proteins antioxidant capacity.

There are many kinds of oxygen radicals produced in the body. The method employed in this study is not necessarily the best model system to assess the ability of a compound to protect against physiologically important reactive oxygen species, such as $O_2\cdot^-$, H_2O_2 and $\cdot OH$. However, insofar as chain-breaking antioxidant activity is concerned, our preliminary studies assess the contribution of some amino acids to the total plasma antioxidant capacity, accounting for the remaining not yet identified plasma antioxidant compounds and supporting that albumin may act as a general pool for the removal of radicals passed in the plasma water.

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