Strand scission in DNA induced by L-DOPA in the presence of Cu(II)

Seema Husain, S.M. Hadi*

Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh-202 002, India

Received 20 March 1995

Abstract **It has been recently reported that L-DOPA and Cu(II) in the presence of H_2O_2 leads to extensive DNA damage. In this paper we show that L-DOPA in the presence of Cu(II) alone can cause DNA cleavage through the generation of reactive oxygen species such as the hydroxyl radical. Fluorescence quenching studies indicate that L-DOPA is capable of binding to DNA.**

Key words: DNA Cleavage; L-DOPA; Copper; Reactive oxygen species

1. Introduction

L-3,4-Dihydroxyphenylalanine (L-DOPA) is an important metabolite in various metabolic reactions. Dopamine, one of the catecholamines, is formed by the decarboxylation of L-DOPA which in turn is formed by hydroxylation of tyrosine. Dopamine is a neurotransmitter in the central nervous system and accounts for 90% of the total catecholamines. It serves as a precursor of hormones, noradrenalin and adrenalin. The neurological disorder Parkinson's disease is associated with an underproduction of dopamine in the human brain [1]. L-DOPA has therefore been found to be an effective drug in the treatment of Parkinson's disease. Another important biochemical reaction for which L-DOPA serves as a metabolite is the synthesis of melanin [2]. Protein-bound 3,4-dihydroxyphenylalanine is a major reductant formed during hydroxyl radical damage to proteins [3].

Copper has been reported to be neurotoxic as evidenced by the brain pathology produced in patients with copper overload as a result of Wilson's disease [4]. Lipid peroxidation is promoted by copper ions [5,6] which also catalyses the formation of highly reactive hydroxyl ($OH^{\cdot}$) radicals from hydrogen peroxide [7]. Copper ions and H_2O_2 produce DNA damage; strand breaks [8] and chemical changes in purine and pyrimidine bases, especially conversion of guanine into 8-hydroxyguanine [9]. Recent work of Halliwell and co-workers [10] has shown that L-DOPA, dopamine and 3-*O*-methyl-DOPA cause extensive base modification in DNA in the presence of H_2O_2 and traces of copper ions. These authors propose that copper ion release, in the presence of L-DOPA and its metabolites, may be an important mechanism of neurotoxicity, e.g. in Parkinson's disease and amyotrophic lateral sclerosis (ALS).

In this paper we describe experiments that show that L-DOPA in presence of Cu(II) alone is capable of causing strand breakage in DNA in vitro and that this breakage results from the generation of reactive oxygen species. We further show that L-DOPA is capable of binding to DNA.

*Corresponding author.

2. Materials and methods

2.1. Materials

Calf thymus DNA (sodium salt, average mol. wt. 1×10^6) and S_1 nuclease were from Sigma (St. Louis, MO). L-DOPA was obtained from Fluka AG, Switzerland. Supercoiled plasmid pBR322 DNA was prepared according to standard methods [11]. All other chemicals were of analytical grade.

2.2. Reaction of L-DOPA with calf thymus DNA and digestion with S_1 nuclease

Reaction mixtures (0.5 ml) contained 10 mM Tris-HCl (pH 7.5), 10 mM NaCl, 500 μg of DNA and varying amounts of L-DOPA and cupric chloride. Free radical scavengers were included in some experiments. Incubation at room temperature was normally for 2 h. All solutions were sterilized before use. S_1 nuclease digestion was performed as described previously [12]. Acid-soluble deoxyribonucleotides were determined either colourimetrically [13] or by determining the absorbance at 260 nm.

2.3. Reaction of L-DOPA with plasmid pBR322 DNA

Reaction mixtures (30 μl) contained 10 mM Tris-HCl (pH 7.5), 0.34 μg plasmid DNA and other components as above. Incubation at room temperature was for 1 h. After incubation, 10 μl of a solution containing 40 mM EDTA, 0.05% Bromophenol blue tracking dye and 50% (v/v) glycerol was added and the solution was subjected to electrophoresis on 1% agarose gels. The gels were stained with ethidium bromide (0.5 mg/l), viewed and photographed on a transilluminator.

2.4. Assay of hydroxyl radicals

Hydroxyl radicals were assayed by incubating a solution containing 2.0 mM salicylate and 15 mM potassium phosphate buffer, pH 8.0, L-DOPA, cupric chloride and other components in a total volume of 2.0 ml for 2 h at room temperature. Salicylate was converted to hydroxylated products, which were extracted and determined colourimetrically [14].

3. Results

3.1. Breakage of calf thymus DNA and cleavage of plasmid DNA by L-DOPA and Cu(II)

L-DOPA and Cu(II) generated S_1-sensitive sites in calf thymus DNA [12]. The reaction was assessed by recording the proportion of double-stranded DNA converted to acid-soluble nucleotides by S_1 nuclease. Increasing concentrations of L-DOPA led to a progressive increase in the production of acid soluble material. The reaction was also dependent on Cu(II) concentration and the time dependency of the reaction was roughly linear up to 2 h (results not shown). Supercoiled pBR322 DNA was examined as a substrate as the relaxation of such a molecule is a sensitive test for just one nick per molecule. L-DOPA converted supercoiled DNA to relaxed open circles and linear forms in a copper-dependent reaction, and at higher concentrations the molecules were converted to progressively smaller heterogenous-sized fragments (Fig. 1A). Similar results were obtained with increasing Cu(II) concentration. Of the several metal ions (Cu(II), Fe(II), Ni(II), Co(II), Mn(II) and Fe(III)) tested, only Cu(II) and to a lesser extent

SSDI 0014-5793(95)00365-7

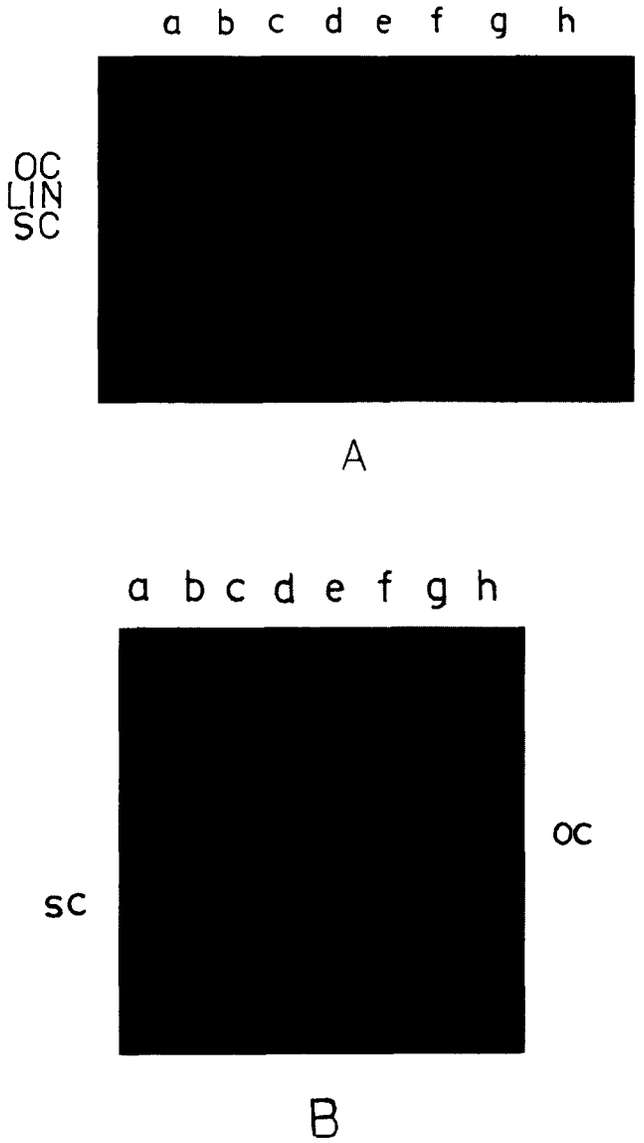


Fig. 1. (A) Agarose gel electrophoretic pattern of ethidium bromide stained pBR322 DNA after treatment with L-DOPA and Cu(II). Lane a, DNA alone; lane b, DNA + Cu(II) (150 μM). Lanes c–h, 25, 50, 75, 100, 150 and 200 μM L-DOPA in the presence of 150 μM Cu(II). (B) Agarose gel elecrophoretic pattern of ethidium bromide stained pBR322 DNA after treatment with L-DOPA and transition metal ions using 20 μM L-DOPA and 20 μM metal ions solutions. Lane a, DNA alone; lane b, DNA + L-DOPA. Lanes c–h, Cu(II), Fe(II), Ni(II), Co(II), Mn(II) and Fe(III), respectively, in the presence of L-DOPA. Reaction mixtures were incubated at room temperature for 1 h. The positions of supercoiled (SC), linear (LIN) and open circular relaxed (OC) DNA are indicated.

Fe(II) complemented L-DOPA in the DNA breakage reaction (Fig. 1B).

3.2. Involvement of active oxygen species in the reaction

The L-DOPA-Cu(II) DNA breakage reaction was inhibited by various radical scavengers (Table 1). Potassium iodide, sodium benzoate, thiourea and mannitol are scavengers of hydroxyl radicals, sodium azide is a singlet oxygen scavenger, whereas superoxide dismutase and catalase remove superoxide anion and hydrogen peroxide, respectively. Except for superoxide dismutase and sodium azide, all the scavengers showed almost maximum inhibition, thereby indicating the essential role of hydrogen peroxide and hydroxyl radicals.

3.3. Production of hydroxyl radicals by L-DOPA

We demonstrated that L-DOPA generates hydroxyl radicals in the presence of Cu(II) (Fig. 2). The assay involves incorporation of salicylate as a reporter molecule [14]. In earlier reports we have shown that this assay genuinely measures hydroxyl radical production as the production is inhibited by thiourea, benzoate and mannitol [15].

3.4. L-DOPA–DNA interaction

When a solution of L-DOPA is excited at 280 nm it exhibits a fluorescence emission spectrum with a maximum around 320 nm [3]. The addition of DNA to L-DOPA causes a decrease in fluorescence emission, and a decreasing degree of fluorescence is seen with increasing concentrations of DNA. In the experiment shown in Fig. 3A, the emission spectra of L-DOPA are given using 8 μM L-DOPA and mixtures with 40, 80, 160, 320, 480, 640 and 800 μM bp calf thymus DNA. The data were also analyzed by Scatchard analysis according to Levine [16] and a plot is shown in Fig. 3B. The plot shows a binding capacity of 2.62 (mol of L-DOPA bound per mol of DNA bp) and a binding affinity (K_a) of 2.64×10^3 l/mol was calculated.

4. Discussion

DNA damage by hydroxyl radicals is well established [17] and the present results indicate a similar mechanism of DNA cleavage by the L-DOPA–Cu(II) system. The hydroxyl radicals are possibly generated through the Fenton reaction which would use hydrogen peroxide and Cu(I) to produce the radical

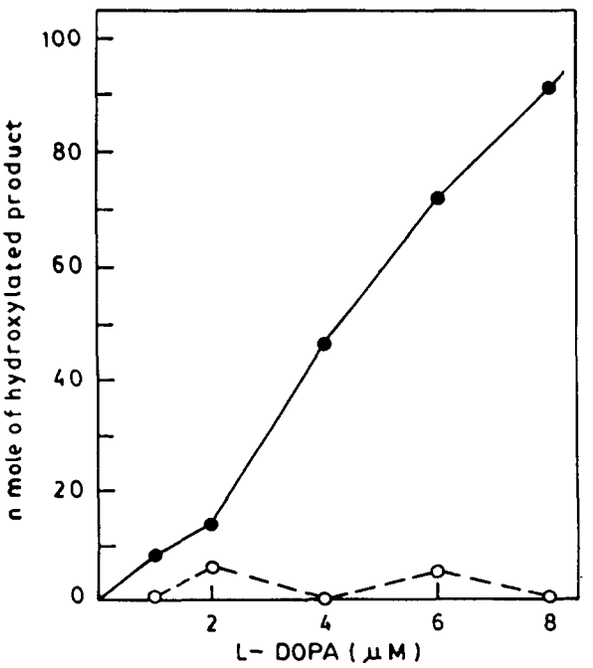
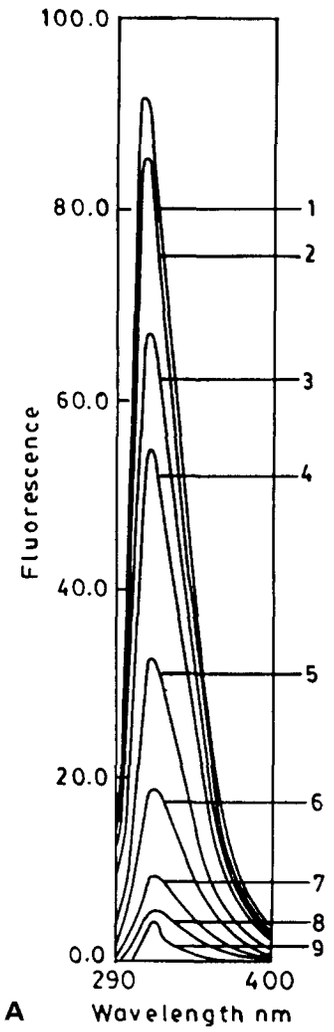


Fig. 2. Formation of hydroxyl radicals as a function of L-DOPA concentration. (●) L-DOPA in presence of 10 μM Cu(II); (○) in the absence of Cu(II). The reaction mixtures were incubated for 2 h at room temperature.



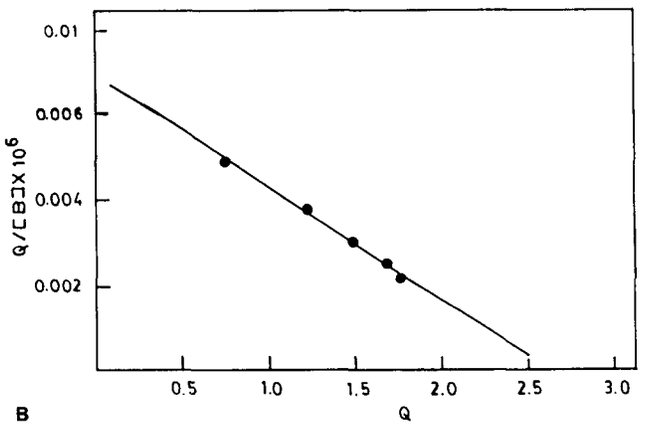


Fig. 3. (A) Effect of calf thymus DNA on the fluorescence emission (excitation wavelength 280 nm) of L-DOPA. Trace 1 is the emission spectrum of L-DOPA alone (8 μM); traces 2–8 are in the presence of DNA at L-DOPA:DNA bp molar ratio of 1:5, 1:10, 1:20, 1:40, 1:60, 1:80 and 1:100, respectively; trace 9 is the emission spectrum of DNA alone. (B) Scatchard plot of the binding of L-DOPA to DNA. The plot was obtained by using the data of A according to Levine [16] where Q is the fractional quench and [B] represents the concentration of unbound ligand.

Table 1
Inhibition of S_1 nuclease hydrolysis of DNA after treatment with L-DOPA (100 μM) and Cu(II) (100 μM) in the presence of scavengers

Scavengers	Inhibition of S_1 nuclease hydrolysis (%)
Catalase (100 μg/ml)	97.0
Superoxide dismutase (100 μg/ml)	7.0
Sodium azide (50 mM)	34.0
Sodium benzoate (50 mM)	86.4
Potassium iodide (50 mM)	90.0
Thiourea (50 mM)	100.0
Mannitol (50 mM)	80.5

Concentration of scavengers shown are final reaction concentrations.

and Cu(II). Thus, presumably Cu(II) is first reduced to Cu(I) in the reaction. There is considerable evidence that reactive oxygen species (ROS), such as the hydroxyl radical, may be important in mutagenesis and carcinogenesis [18,19]. ROS can be generated within cells by several metabolic processes such as those involving redox enzymes and bioenergetic electron transfer. They may also be produced by many known chemical mutagens either alone or in association with some transition metal ions [20–22]. In addition, the mechanism of action of several anti-tumor antibiotics such as bleomycin and adriamycin [23,24] is considered to involve the generation of ROS. Several known antioxidants of plant origin such as flavonoids, generate ROS in the presence of transition metal ions such as copper, and lead to DNA cleavage [12,15,25]. A number of molecules present in human extracellular fluids are considered to have antioxidant function and these include ascorbic acid and uric acid. Production of hydroxyl radical and DNA damage by ascorbate and H_2O_2 in the presence of Cu(II) has been reported [26,27]. We have recently shown that uric acid in the presence of Cu(II) alone is capable of causing strand scission in DNA and that this reaction is associated with generation of ROS [28]. We have therefore suggested that several of the biological antioxidants are themselves capable of generating ROS under appropriate conditions. In view of this and the fact that L-DOPA is produced from tyrosine by a copper-containing enzyme, namely tyrosine hydroxylase, and presents a possibility of copper chelation, we considered it of interest to explore the generation of ROS and DNA damage by a L-DOPA and Cu(II) system. In the light of recent work of Halliwell et al. [10] the present results further emphasize the putative role in vivo of L-DOPA and copper ions as a source of oxidative DNA damage. It is recognized by most workers that hydroxyl radical reactions with DNA are preceded by the association of a complex with DNA, followed by the production of hydroxyl radicals at that particular site [29]. As shown above, L-DOPA is capable of binding to DNA, and if copper ions are available a ternary complex of L-DOPA-Cu(II)-DNA may be formed as in the case of several other ROS generating and DNA cleavage systems [12, 30]. Halliwell and co-workers [10] have shown that copper ion concentrations in human brain tissue damaged in Parkinson's disease are at a level that could promote oxidative DNA damage.

References

[1] Lehninger, A.L., Nelson, D.L. and Cox, M.M. (1993) Principles of Biochemistry, Worth Publishers, USA.

[2] Hill, H.Z. (1992) Bioassays 1, 49–53.
[3] Gieseg, S.P., Simpson, J.A., Charlton, T.S., Duncan, M.W. and Dean, R.T. (1993) Biochemistry 32, 4780–4786.
[4] Hartard, C., Weisner, B., Dieu, C. and Kunze, K. (1993) J. Neurol. 241, 101–107.
[5] Hochstein, P., Kumar, K.S. and Forman, S.J. (1980) Ann. NY Acad. Sci. 355, 240–248.
[6] Esterbauer, H., Striegl, G., Puhl, H., Rotheneder, M. (1989) Free Rad. Res. Commun. 6, 67–75.
[7] Halliwell, B. and Gutteridge, J.M.C. (1990) Methods Enzymol. 186, 1–85.
[8] Tachon, P. (1990) Free Rad. Res. Commun. 9, 39–47.
[9] Aruoma, O.I., Halliwell, B., Gajewski, E., Dizdaroglu, M. (1991) Biochem. J. 273, 601–604.
[10] Spencer, J.P.E., Jenner, A., Aruoma, O.I., Evans, P.J., Kaur, H., Dexter, D.T., Jenner, P., Lees, A.J., Marsden, D.C., Halliwel, B. (1994) FEBS Lett. 353, 246–250.
[11] Maniatis, T., Fritsch, E. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[12] Rahman, A., Shahabuddin, Hadi, S.M., Parish, J.H. and Ainley, K. (1989) Carcinogenesis 10, 1833–1839.
[13] Schneider, W.C. (1957) Methods Enzymol., 3, 680–684.
[14] Richmond, R., Halliwell, B., Chauhan, J. and Darbre, A. (1981) Anal. Biochem. 118, 328–335.
[15] Fazal, F., Rahman, A., Greensill, J., Ainley, K., Hadi, S.M. and Parish, J.H. (1990) Carcinogenesis 11, 2005-2008.
[16] Levine, R.L. (1977) Clin. Chem. 12, 2292–2301.[17] Halliwell, B. and Aruoma, O.I. (1991) FEBS Lett. 281, 9–19.
[18] Feig, D.I. and Loeb, L.A. (1993) Biochemistry 32, 4466–4473.
[19] Imlay, J.A. and Linn, S. (1988) Science 240, 1302–1309.
[20] Ames, B.N. (1983) Science 221, 1256–1264.
[21] Hsie, A.W., Xu, Z., Yu, Y., Sognier, M.A. and Hrelia, P. (1990) Teratog. Carcinog. Mutagen. 10, 115–124.
[22] Kasprzak, K.S. (1991) Chem. Res. Toxicol. 4(6), 604–615.
[23] Ehrenfeld, G.M., Shipley, J.B., Heimbrook, D.C., Sugiyani, H., H., Long, E.C., Van Boom, J.H., Vander Marel, G.A., Oppenheimer, N.J. and Hecht, S.M. (1987) Biochemistry 26, 931–942.
[24] Eliot, H., Gianni, L. and Myers, C. (1984) Biochemistry 23, 928–936.
[25] Said Ahmed, M., Ramesh, V., Nagaraja, V., Parish, J.H. and Hadi, S.M. (1994) Mutagenesis 3, 193–197.
[26] Rowley, D.A. and Halliwell, B. (1983) Arch. Biochem. Biophys. 225, 279–284.
[27] Stoewe, R. and Perutz, W.A. (1987) Free Rad. Biol. Med. 3, 97–105.
[28] Shamsi, F.A. and Hadi, S.M. (1995) Free Rad. Biol. Med. (in Press).
[29] Pryor, W.A. (1988) Free Rad. Biol. Med. 4, 219–223.
[30] Wong, A., Huang, C.H. and Crooke, S.T. (1984) Biochemistry 23, 2946–2952.