Review

Methodology for Measuring Lipid Peroxidation in Biological Materials : A Bibliography

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ABSTRACT

Various substances have been found and used as markers of different stages of lipid peroxidation reaction. Free radicals detection would provide more meaningful information when the method is used in clinical studies involving living cells or tissue biopsy. Lipid oxidation products may be used as markers of lipid peroxidation, especially in food products. Each method currently available for measuring the extent of lipid peroxidation should be used precautiously and its limitations should be anticipated.

INTRODUCTION

Lipid peroxidation is one major cause of quality deterioration in foods, especially during prolonged storage at room temperature. In addition, peroxidized lipid and its various degradation products has been reported to cause tissue injury in animal studies. Research progress in these areas, however, is relatively slow. This, in part, is because most of the existing methods are still unspecific, insensitive and time consuming. Therefore, every selected method should be used with precaution and results from the analysis should be interpreted correctly.

Initiation of a peroxidation reaction sequence of polyunsaturated fatty acids (PUFA) in biological materials is due to attack by any free radical that has sufficient reactivity such as hydroxyl ($OH^{\bullet}$), alkoxyl ($RO^{\bullet}$) and alkylperoxyl ($ROO^{\bullet}$) radicals which abstract a hydrogen atom from a methylene ($-CH_2-$) group. Since a hydrogen atom has only one electron, this leaves behind an unpaired electron on the carbon. The presence of a double bond in the fatty acid weakens the C-H bonds on the carbon atom adjacent to the double bond and so makes the removal of hydrogen atoms with one electron easier. The remaining carbon radical tends to be stabilized by a molecular rearrangement to produce a conjugated diene, which then easily reacts with an oxygen molecule to give an alkylperoxyl radical. This radical can abstract a hydrogen atom from another PUFA molecule to form a lipid hydroperoxide (ROOH) (Halliwell and Gutteridge, 1986). In the presence of transition metals (Fe^{2+} or Cu^{2+}), the lipid hydroperoxide is easily broken down to alkoxyl or alkylperoxyl radicals, and other secondary products such as ethane, pentane, malonaldehyde, hexanal and 4-hydroxynonenal (Gutteridge and Halliwell, 1990). Therefore, the extent of lipid peroxidation in biological materials can be measured at different stages, involving detection of free radicals, lipid hydroperoxides and their degradation products, oxygen uptake, and loss of PUFA.

The objective of this paper is to conduct a bibliography study on methodology for measuring lipid peroxidation in biological materials.

METHODS FOR MEASURING LIPID PEROXIDATION

The primary products of lipid peroxidation are lipid hydroperoxides, which themselves are strong oxidizing agents and can easily react with amino acids and proteins (Gardner, 1979). In the presence of transition metal ions the lipid hydroperoxides are easily degraded into secondary products such as aldehydes, ketones and hydrocarbons, and other hydroperoxyl or alkoxyl radicals (Mead et al., 1986; Kanner et al., 1988; Thomas and Aust, 1989; Kanner and Doll, 1991). These primary and secondary products have a direct effect on objectionable flavors or odors present in rancid vegetable oils and fats (Selke et al., 1980; Frankel et al., 1981; Selke and Frankel, 1987; Przybylski and Hougen, 1989), in model systems using PUFA esters (Pitkanen et al., 1989; Hau and Nawar, 1988; Ullrich and Grosch, 1988;

Frankel et al., 1989), in rancid snack food (Boggs et al., 1964; Bengtsson et al., 1967; Fritsch and gale, 1977; Min and Schweizer, 1983; Robards et al., 1988) and in rancid meat products (Angelo et al., 1987; 1988).

In addition to undesirable odors, products of lipid peroxidation may alter the functional properties of myofibrilar proteins (Smith et al., 1990) as well as lower the nutritive value of oxidized food products (Hurrell and Nielsen, 1987). Evidence also exists that lipid peroxidation products have the potential of inducing mutagenic activity in biological systems (Hagemen et al., 1988; 1989; Vaca and Harms-Ringdahl, 1989a; 1989b; Brambilla et al., 1989; Esterbauer et al., 1990). Particularly important is considered the potential for cytotoxicity by malonaldehyde (Nair et al., 1986; Masaki et al., 1989).

Unfortunately, there is no single method which satisfactorily measures the overall process of lipid peroxidation in all types of biological materials. Methods of direct measurement of lipid hydroperoxides by enzymatic reaction such as, cyclooxygenase, glutathione peroxidase, hemoglobin peroxidase and horseradish peroxidase activity (Table 1), seem to be sensitive and specific. However, these methods may be applicable only in limited types of substrates, such as fatty acid hydroperoxides which usually must first be cleaved from their primary molecules sites such as phospholipids or triacylglycerides. Hemoglobin peroxidase reacts with phosphatidylcholine hydroperoxides to a lesser extent that fatty acid hydroperoxides (Shibata et al., 1986). Since hemoglobin is used in the hemoglobin peroxidase method, it is likely to interfere with hemoglobin present in samples such as meat (Thomas and Poznansky, 1990). Another limitation of enzymatic methods is that the lipid hydroperoxides need to be recovered from complex biological matrices. Because of the instability of lipid hydroperoxides, it is likely that these hydroperoxides degrade easily into secondary products or react with other nonlipid substances during preparation.

Generation of oxygen radicals has been associated with the onset of free radical-induced tissue injuries (Slater, 1984b; Fuller et al., 1988), diseases (Halliwell and Gutteridge, 1984; Golden and Ramdath, 1987; Clark et al., 1989; Doelman and Bast, 1990), and carcinogenesis (Sun, 1990; Floyd, 1990; Babbs, 1990; Trush and Kensler, 1991). In these circumstances, detection of free radicals (Forman and Borg, 1989, McCay and Poyer, 1989; Jackson and Johnson, 1989) should provide more meaningful information on the relationship between the early state of lipid peroxidation and the progression of disease processes.

In studies of meat processing and storage of finished meat products the extent of lipid peroxidation is followed for several days, weeks, months or even years. In addition, many of these meat products are precooked. Heating treatments (Salih et al., 1989; Fogerty et al., 1990, Lyon and Ang, 1990) and prolonged storage, even under refrigeration (Tomas and Funes, 1987; Lyon et al., 1988; Dawson et al., 1990) or freezing temperatures (McNeill et al., 1987; Barbut et al., 1988; Tomas and Anon, 1990), favor degradation of lipid hydroperoxides into secondary products. Under these circumstances, detection of malonaldehyde, hexanal, 4-hydroxynonenal and other degradation products (Table 1) in meat products is more meaningful and practical than detection of free radicals.

SUMMARY

It is obvious that various markers can be used in the measurement of different stages of lipid peroxidation reactions. Free radicals detection can provide more relevant information when the method is used in clinical studies involving living cells or tissue biopsy. Various lipid oxidation products can be used as markers of lipid peroxidation, especially in food products. Since every method for measuring the extent of lipid peroxidation has its own advantages and limitations, users shoulds well aware about them prior to selection.

Table 1. **Methods for measurement of lipid peroxidation in biological materials**

Compound detected	Method of detection	Remarks and references
Free radicals	Electron spin resonance	Spin trapping is required to extend the life time of or free radicals in biological materials (Janzen, 1980; Lunec, 1989; Pou et al., 1989; Chamulitrat and Manson, 1989; Lunec and Griffiths, 1990; Xu et al., 1990; Pou and Rosen, 1990, Buettner and Mason, 1990; Evans and Jackson, 1990)

Table 1. (continued)

Compound detected	Method of detection	Remarks and references
Free radicals	Chemiluminescence	Luminol is used to amplify chemiluminescent signals; applicable for intact organs or tissue analysis (Boveris et al., 1980, Cadenas and Sies, 1984; Murphy and Sies 1990).
Conjugated diene	Spectrophotometric	Rearrangement of the PUFA double bonds leads to the formation of conjugated dienes (Buege and Aust, 1978). Further modifications by Corongiu and Milia (1983), and Corongiu et al., (1989).
Lipid peroxides	Iodometric	Iodide ion reduces fatty acid hydroperoxides into hydroxy fatty acids (Buege and Aust, 1978); more sensitive methods have been developed (Takagi et al., 1978; Hara and Totani, 1988; Gebicki and Guille, 1989; Thomas et al., 1989).
Lipid peroxides	Cyclooxygenase activity	Fatty hydroperoxide activates the cyclo-oxygenase activity of prostaglandin H synthase (Marshall et al., 1985; Kulmacz et al., 1990).
Lipid peroxides	Glutathione peroxidase	Fatty hydroperoxide reacts with glutathione (GSH) catalyzed by GSH peroxidase to produce glutathione disulfide (GSSG) (Heath and Tappel, 1976; Allen et al, 1990).
Lipid peroxides	Hemoglobin peroxidase	Leucodye is used as a hydrogen donor and the color of its oxidized form can be measured spectrophotometrically. It is limited for fatty acid hydroperoxides (Shibata et al., 1986; Matsushita et al., 1987).
Lipid peroxides	Dichlorofluorescein fluorescent	Nonfluorescent dichlorofluorescin is oxidized by hydrogen peroxide to produce fluorescent dichlorofluorescein (Keston and Brandt, 1965; Cathcart et al., 1984; Ferrer et al., 1990).
Lipid peroxides	Horseradish peroxidase	The principle is similar to the hemoglobin peroxidase (Yamaguchi, 1980).
Lipid peroxides	Chemiluminescence and HPLC	Luminol is oxidized the emits chemiluminescence detected with HPLC (Yamamoto and Ames, 1987; Miyazawa et al., 1987, Iwaoka et al., 1987; Miyazawa, 1989; Yamamoto et al., 1990).
Lipid peroxides	Fluorometric HPLC	Diphenyl-1-pyrenylphosphine (DPPP) reacts with lipid hydroperoxide to produce fluorescent DPPP-oxide (Meguro et al., 1990).
Lipid peroxides	GC-MS	Fatty acid hydroperoxide is reduced by triphenylphosphine to form hydroxy acid which can be identified and quantified by GC-MS (Hughes et al., 1983; 1986; Smith and Anderson, 1987; van Kuijk et al., 1990a).

Table 1. (continued)

Compound detected	Method of detection	Remarks and references
Lipid peroxides	^{13}C-NMR Spectroscopy	It can be used for quantitative analysis of positional and geometric isomers of linoleate hydroperoxide (Frankel et al., 1990). ^{1}H-NMR was used by Lamba et al. (1991); Saito and Nakamura (1990).
Lipid peroxides	Sesamol dimer	Lipid peroxides react with hemoglobin to form peroxyl radicals which oxidize colorless sesamol dimer (I) into violet color of sesamol dimer quinone (II) (Kurechi et al., 1981; Kikugawa et al., 1983; 1985; Kikugawa, 1989).
Losses of PUFA	GC	Polyunsaturated fatty acids are oxidized and decomposed into degradation products (Kim and LaBella, 1987; Pompella et al., 1987; Barbut et al., 1989).
4-hydroxynonenal	GC-MS	4-hydroxynonenal is reacted with o-penta-fluorobenzylhyxroxylamine to form o-penta-fluorobenzyl oxime (van Kuijk et al., 1990b).
4-hydroxynonenal	GC	4-hydroxynonenal is reacted with N-methyl-hydrazine to produce 5 (l'-hydroxyhexyl)-l-methyl-2-pyrazoline (Dennis and Shibamoto, 1990; Tamura and Shibamoto, 1991).
4-hydroxynonenal	HPLC	4-hydroxynonenal is reacted with 2,4-dinitrophenylhydrazine to form dinitrophenyl-hydrazone derivative (Esterbauer and Zollner, 1989; Esterbauer and Cheeseman, 1990)
Oxygen uptake	GC or Oxygen probe	Oxygen is consumed by alkyl radical to produce alkylperoxyl radical (Min and Schweizer, 1983; Fioriti, 1977; Slater, 1984a; 1984b).
Pentanal, hexanal, ethane and pentane	Headspace GC	Samples are incubated in a closed container at elevated temperature to generate these volatile compounds in the headspace (Angelo et al., 1987; Robards et al., 1988; Ang and Young, 1989; Frankel et al., 1989; Przybylski and Hougen, 1989; Logliger, 1990).
Malonaldehyde	HPLC	It can be performed without derivatization (Kakuda et al., 1981; Csallany et al., 1984; Bull and Marnett, 1985; Largilliere and Melancon, 1988; Lee and Csallany, 1987). Malonaldehyde derivatized into l-dansyl-pyrazole (Hirayama et al., 1984) or derivatized into pyrimidine compound (Kishida et al., 1990).
Malonaldehyde-TBA complex	HPLC	Malonaldehyde is reacted with TBA to form malonaldehyde-TBA complex (Bird et al., 1983; Bird and Draper, 1984; Wong et al., 1987; Yoden and Iio, 1989; Tatum et al., 1990; Draper and Hadley, 1990; Squires, 1990).

Table 1. (continued)

Compound detected	Method of detection	Remarks and references
Malonaldehyde	GC	Malonaldehyde is reacted with 2-hydrozino-benzothiazole (HBT) to form HBT-malonaldehydè condensation (Beljean-Leymarie and Bruna, 1988), with methylhydrazine to form l-methylpyrazole (Umano et al., 1988; Ichinose et al., 1989; Dennis and Shibamoto, 1989a; Tamura et al., 1991) with urea to form 2-hydroxypyrimidine (Hamberg et al., 1968), with 1,3-propanediol to form malonaldehydebis-1,3-dioxane (Lakshminarayana and Cornwell, 1986), or with pentafluorophenylhydrazine (PFPH) to form malonaldehyde-PFPH complex (Tomita et al., 1990).
Malonaldehyde-TBA complex	GC	The malonaldehyde-TBA complex may not be completely resolved from the non-malonaldehyde-TBA complex (Dennis and Shibamoto, 1989a; 1989b).
Malonaldehyde	Spectrofluorometry	It has better specificity and sensitivity than TBA test with spectrophotometry (Sawicki et al., 1963; Kikugawa and Sugimura, 1986; Iwata and Kikugawa, 1987; Kikugawa et al., 1988; Hirayama et al., 1990).
Malonaldehyde-TBA complex	Spectrofluorometry	It has better sensitivity than TBA test with spectrophotometry (Yagi, 1976; 1989).
Malonaldehyde	Polarography	It is specific for free malonaldehyde (Bond et al., 1980).
Malonaldehyde-TBA complex	Spectrophotometry	It is a simple and quick test, but it may be insensitive and not adequately specific (Bird et al., 1983; Hackett et al., 1988; Wade and van Rij, 1989; Gutteridge and Halliwell, 1990; Kojima et al., 1990; Squires, 1990). The use of C_{18} (octadecylsilane) cartridge improves the specificity and sensitivity of the TBA test (Raharjo et al., 1993).

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