Lipid peroxidation

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Lipid peroxidation is a complex process, involving free-radical intermediates, whereby unsaturated fatty acids (and some other types of unsaturated lipid) are oxidatively degraded to a variety of products. The control of lipid peroxidation is of special significance commercially to the oil and food industries; in biology, the peroxidation of polyunsaturated fatty acids, especially arachidonic acid, is of particular importance in relation to membrane damage. General reviews of the biological features and consequences of lipid peroxidation are by Slater (1975), Mead (1976), Dianzani & Ugazio (1978) and by Simic & Karel (1980).

Lipid peroxidation of unsaturated fatty acids involves an initiation step to yield the fatty acid free radical, followed by propagation steps involving the addition of dioxygen to form peroxy radicals and hydroperoxides. Very-electrophilic oxidizing radicals such as $OH^{\bullet}$ can H-abstract from any of the non-carboxylate carbons of a fatty acid such as arachidonic acid (AA), for example, but the methene hydrogens in divinyl methane structures, and allylic hydrogens in general are the most susceptible (Hasegawa & Patterson, 1978) to less-reactive radicals. The radical $R^{\bullet}$ required for such an initiation event can be formed in the vicinity of the fatty acid by radiation, or by metabolic activation. The peroxy radicals and peroxides formed during propagation steps can undergo extensive degradation, especially in the presence of iron and copper ions to give a complex mixture of products including ketones, esters, aldehydes, alkenes and alkanes (Gardner, 1975). For background references on these degradation products, see Schauenstein (1967) and Esterbauer (1982).

Scheme 1 illustrates the above remarks and indicates methods that have been used in monitoring the course of lipid peroxidation; the only method for studying lipid peroxidation under non-invasive conditions *in vivo* is the measurement of pentane or ethane in the expired air (Riely *et al.*, 1974). However, with the latter technique it is difficult to be sure of the tissue, cellular and/or intracellular origin of the alkane products. Another technique used for studying lipid peroxidation in the intact organ *in situ* has been chemiluminescence (Boveris *et al.*, 1980).

(i) $R^{\bullet} + AA \rightarrow RH + AA^{\bullet}$
(ii) $AA^{\bullet} \rightarrow AA^{\bullet}$ (**diene conjugation** rearrangement)
(iii) $AA^{\bullet} + O_2 \rightarrow AAO_2^{\bullet}$
(iv) $AAO_2^{\bullet} + AA \rightarrow AAO_2H + AA^{\bullet}$
(v) $AA^{\bullet}, AAO_2^{\bullet} \rightarrow$ non-radical products
(vi) $AAO_2^{\bullet}, AAO_2H \rightarrow$ alkanes (**ethane**, **pentane**, etc). alkanals, alkenes, alkenals, **malonaldehyde**, 4-hydroxy alkenals

Scheme 1. *Abbreviated scheme of lipid peroxidation involving a chemically reactive radical ($R^{\bullet}$) and arachidonic acid (AA)*

The initiation step involving hydrogen abstraction by $R^{\bullet}$ is shown in (i); propagation steps are (iii) and (iv); chain termination steps are shown in (v). Methods used for monitoring peroxidation have used estimations of components shown in **bold** type: in (i) disappearance of the parent AA; in (ii) rearrangement of the double bonds in $AA^{\bullet}$ to give diene conjugation with increased absorption at 232nm; in (iii) oxygen consumption; in (iv) production of lipid hydroperoxide by iodometric or enzyme-assay procedures; in (vi) evolution of hydrocarbon gases and production of malonaldehyde. Background references to these techniques can be obtained from Beswick *et al.* (1982). In addition, chemiluminescence has been used for studying lipid peroxidation (see Guthaus *et al.*, 1979; Wright *et al.*, 1979).

Under conditions *in vitro*, most studies on lipid peroxidation have been done with rat liver microsomes, and by using the thiobarbituric acid (TBA) reaction for malonaldehyde estimation, diene-conjugation measurement, or the loss of polyunsaturated fatty acid. The TBA reaction correlates quite closely with chemiluminescence during short incubation times, with O_2 consumption and with loss of arachidonic acid in rat liver microsomes (Beswick *et al.*, 1982). Although the TBA reaction has been criticized on occasions as being non-specific for malonaldehyde, recent studies using high-pressure liquid chromatography have demonstrated a very good correlation between the TBA reaction and malonaldehyde content (Esterbauer & Slater, 1981).

Lipid peroxidation can be stimulated in rat liver microsomes by the addition of NADPH, ascorbate or cumene hydroperoxide. NADPH-mediated peroxidation is enzymically catalysed by NADPH:cytochrome *P*-450 reductase, whereas cumene hydroperoxide-mediated peroxidation involves cytochrome *P*-450 itself acting as a peroxidase (O'Brien & Rahimtula, 1975). Ascorbate-linked microsomal peroxidation is largely non-enzymic. The NADPH-mediated peroxidation can be substantially increased by the addition of iron chelates (e.g. ADP/Fe^{2+}; Hochstein & Ernster, 1963), or of carbon tetrachloride (see Slater, 1982*a*,*d*). In NADPH-mediated or carbon tetrachloride-stimulated lipid peroxidation, microsomes from liver were much more active than microsomes prepared from other rat tissues (Benedetto *et al.*, 1981).

A considerable number of toxic substances are now known to be metabolically activated by the liver NADPH:cytochrome *P*-450 system to give reactive free-radical intermediates (see Mason, 1979; Slater, 1982*b*) that can initiate lipid peroxidation. The latter event can be a significant feature of the overall cell damage or cell death resulting from exposure to the toxic agent. Some examples of substances that are metabolically activated to free-radical products and which cause a stimulation of peroxidation, are carbon tetrachloride (Recknagel & Glende, 1973; Slater, 1982*a*), quinones like adriamycin and daunorubicin (Goodman & Hochstein, 1977; Lown, 1982), paracetamol (Wendel *et al.*, 1979; De Vries, 1981). In such situations cytochrome *P*-450 may act in catalysing the formation of the initiating radical $R^{\bullet}$ and in propagating the peroxidative chain through its peroxidase-type of activity (Svingen *et al.*, 1979).

Lipid peroxidation is generally decreased in tumour tissues compared with the corresponding normal tissues (Barber & Bernheim, 1967; Ugazio *et al.*, 1968), and this has been suggested to result from the occurrence, in tumour fractions, of a high concentration of anti-oxidant material that can inhibit the free-radical events of lipid peroxidation (Lash, 1966; Arneson *et al.*, 1978). Studies on the regulation and restriction of lipid peroxidation are also important in relation to attenuating the disturbances seen in tissue injury (e.g. by carbon tetrachloride, adriamycin, etc.). Quantitative kinetic data on the reactivity of the activated radical intermediates (e.g. $CCl_3^{\bullet}$) of the fatty acid and peroxy radicals, and the rate of reaction of free-radical scavengers have mostly been obtained by using pulse radiolysis (see Willson, 1978; Slater, 1982*b*). Another useful technique for studying the reactive free-radical intermediates is electron-spin-resonance spin trapping (see Janzen, 1980). Such studies have shown how restricted must be the diffusion of highly reactive intermediates such as $CCl_3O_2^{\bullet}$ (Slater, 1979) and the necessity, therefore, to get a protective scavenger into the right site, at the right time and in a suitable concentration. Criteria for free-radical scavenging in relation to metabolic activation and lipid peroxidation have been discussed by Slater (1982*c*). Other

aspects of protection against the consequences of lipid peroxidation are the reduced glutathione (GSH)-dependent metabolism of lipid peroxides (Christophersen, 1968) and metabolism of aldehydic end products. GSH is also important in relation to direct reactions with aldehydes and ketoaldehydes, and with activated intermediates; a review of such roles for GSH is given by Sies & Wendel (1978) and Akerboom *et al.* (1981).

Although the initiating radical R$^{\bullet}$, and the secondary radicals AA$^{\bullet}$ and $AAO_2^{\bullet}$ are restricted in their radii of diffusion by their relatively higher chemical reactivity, some of the breakdown products of lipid peroxidation display interesting and important biological properties that can spread the effects of a localized peroxidation to more distant sites (see Slater, 1976). Some early products like hydroperoxyeicosatetraenoic acid can cause histamine release when present at very low concentration (Peters *et al.*, 1981); lipid peroxides can affect the prostaglandin cascade and disturb the ratio of the various products ratio (Hemler *et al.*, 1979); aldehydic products can cross-link proteins (Chio & Tappel, 1969), decrease membrane fluidity (Slater, 1979; Dobretsov *et al.*, 1977), affect adenyl cyclase at micromolar concentration (Dianzani, 1982), and cause inhibition of membrane-linked enzymes such as glucose 6-phosphatase (Recknagel & Glende, 1973). In the latter respect the isolation and identification of 4-hydroxynonenal by Benedetti *et al.* (1980) has been most interesting. Very recent studies by Esterbauer *et al.* (1981) have shown how complex is the pattern of alkenals and 4-hydroxy alkenals that is produced during lipid peroxidation. Since some 4-hydroxy alkenals have been shown to have anti-tumour activity *in vivo* (Schauenstein *et al.*, 1977) and specifically react with nuclear thiol groups (Schauenstein, 1982), a new and potentially important aspect of lipid peroxidation is emerging.

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