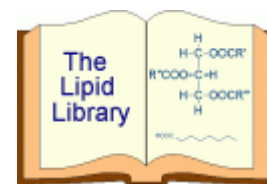
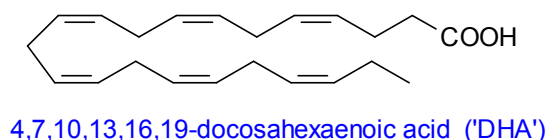


RESOLVINS AND PROTECTINS

Chemistry and Biology

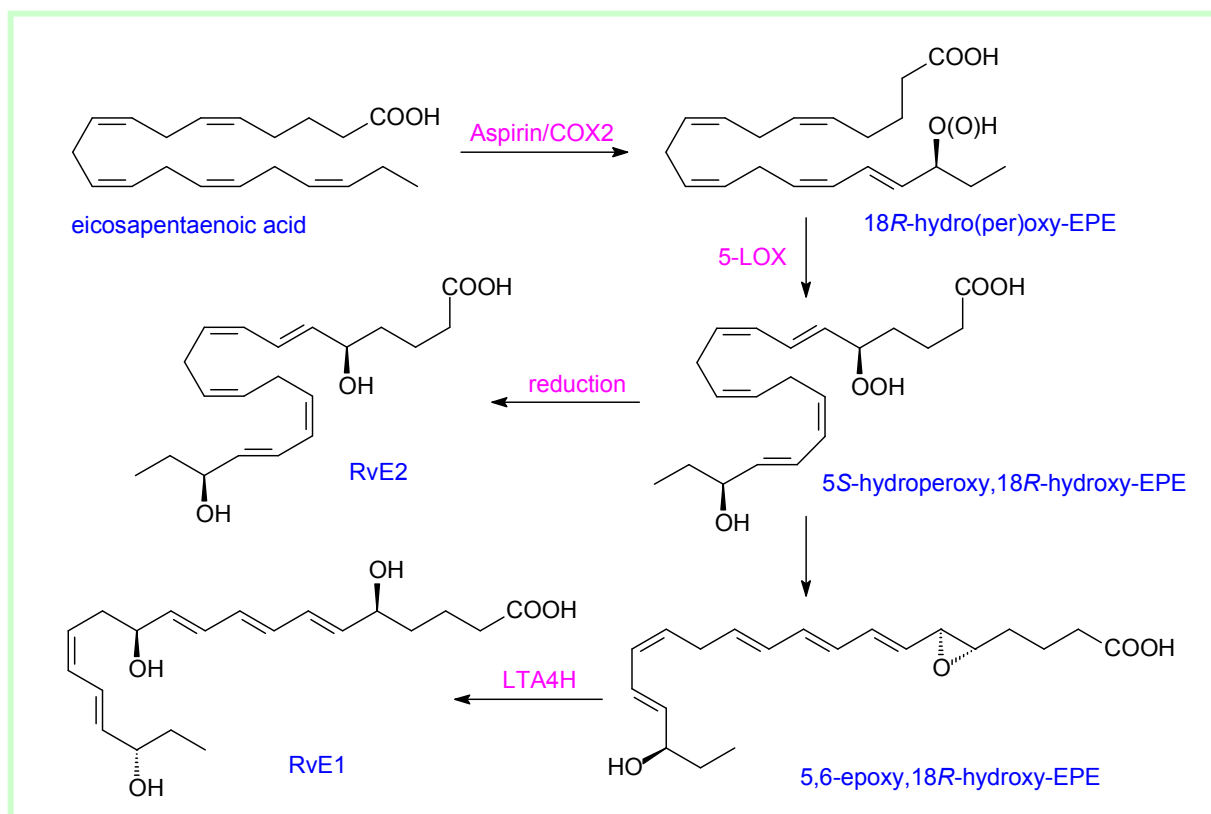


The omega-3 essential fatty acids are the focus of considerable interest among nutritionists, because of the perceived beneficial effects for the health of consumers. The mechanisms by which such effects are exerted is still a matter for controversy, but it seems likely that oxygenated metabolites derived from eicosapentaenoic acid (20:5(n-3) or EPA) and docosahexaenoic acid (22:6(n-3) or DHA), the resolvins and (neuro)protectins, must play a significant part as they have potent anti-inflammatory and immunoregulatory actions at concentrations in the nanomolar and picomolar range. The term '**resolvins**' or '*resolution-phase interaction products*' was coined by Professor Charles N. Serhan and colleagues because these compounds were first encountered in resolving inflammatory exudates. Compounds derived from EPA are designated as resolvins of the E series, while those formed from the precursor DHA are denoted as either resolvins or protectins ('neuroprotectins') of the D series. Increasing numbers of such compounds are being identified and only the more important series are discussed here.



1. 18R Resolvins of the E Series

Biosynthesis of the 18R resolvins of the E series (name derived from EPA) has elements in common with the synthesis of the **epi-lipoxins** and **leukotrienes**.

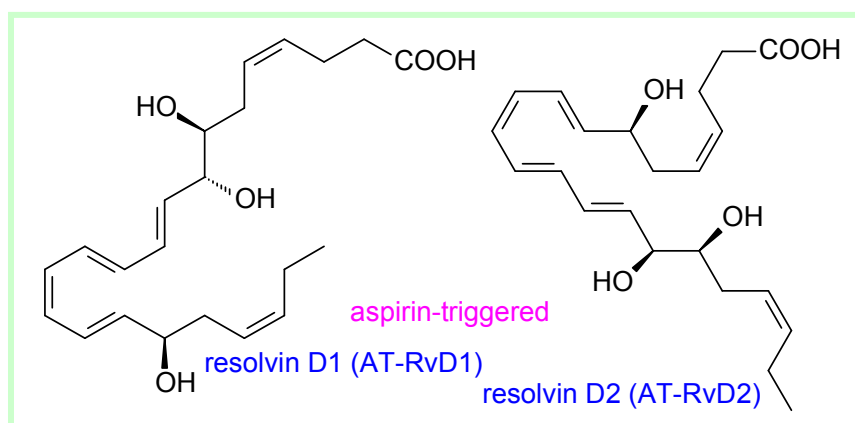


In vascular endothelial cells derived from blood vessels, the cyclooxygenase enzyme COX-2 that has been acetylated by aspirin introduces an 18*R* hydroperoxy-group into the EPA molecule (c.f. the role of aspirin in the biosynthesis of the **epi-lipoxins**). The product is reduced to the corresponding hydroxy compound before a 5*S*-hydroperoxy group is introduced into the molecule by the action of 5-lipoxygenase as in the biosynthesis of the leukotrienes. A further reduction step produces 15*S*,18*R*-dihydroxy-EPE or resolvin E2. Alternatively, the 5*S*-hydroperoxy,18*R*-hydroxy-EPE intermediate is converted to a 5,6-epoxy fatty acid in polymorphonuclear neutrophils in humans and eventually to 5*S*,12*R*,18*R*-trihydroxy-6*Z*,8*E*,10*E*,14*Z*,16*E*-eicosapentaenoic acid or resolvin E1 by an enzyme required for the biosynthesis of leukotrienes in leukocytes. It is noteworthy that the initial oxygenation reaction is not catalysed by unacetylated COX-2 or COX-1.

The highly specific stereochemistry of resolvin E1 is required for activation of a ligand-specific receptor and thence for its biological activity down to picomolar concentrations. However, epimeric 18*S*-resolvins are also produced *in vivo* by related biosynthetic pathways with their own distinctive biological activities, and similarly the precursor 18(*R/S*)-hydroxy-EPE has been shown to have anti-inflammatory effects *in vitro*. Resolvin E1 is eventually de-activated in tissues by oxidation via at least four distinct pathways, including conversion to 18- and 12-oxo-RvE1, for example, prior to further catabolism.

2. 17*R*- and 17*S*-Resolvins of the D Series

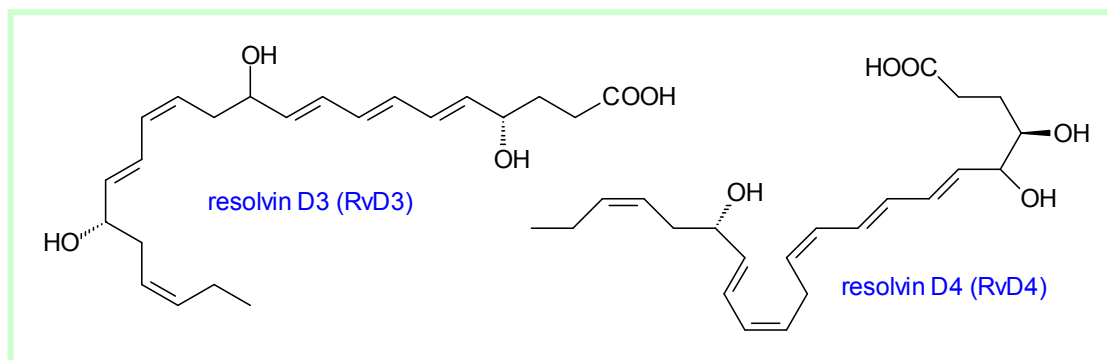
DHA is converted to 17*R*-resolvins by a similar aspirin-triggered COX-2 mechanism to the previous. (In the absence of aspirin, COX-2 in human microvascular endothelial cells converts DHA to 13*S*-hydroxy-DHA). Thence, enzymatic epoxidation generates either 7*S*,(8)-epoxy or a 4*S*,(5)-epoxy intermediate, which are acted upon by 5-lipoxygenase to yield the resolvins. The former produces the aspirin-triggered resolvins D1 and D2 as illustrated (D nomenclature derived from DHA), while the latter produces the aspirin-triggered resolvins D3 and D4. All contain a 17*R* hydroxyl group. Again, there are parallels in the biosynthesis of resolvins (and protectins below) with that of **epi-lipoxins (or aspirin-triggered lipoxins)** from arachidonate.



In an alternative reaction in the absence of aspirin, 15-lipoxygenase generates 17*S*-hydroxy-DHA as the initial product. This is converted to 7*S*-hydroperoxy,17*S*-hydroxy-DHA by the action of a 5-lipoxygenase, and thence via epoxy intermediates to resolvin D1 (RvD1 or 7*S*,8*R*,17*S*-trihydroxy-docosa-4*Z*,9*E*,11*E*,13*Z*,15*E*,19*Z*-hexaenoic acid) and epimeric resolvin D2 (RvD2 or 7*S*,16,17*S*-trihydroxy-docosa-4*Z*,8*E*,10*Z*,12*E*,14*E*,19*Z*-hexaenoic acid), i.e. all contain a 17*S* hydroxyl group.

A further lipoxygenase-generated intermediate from 17*S*-hydroxy-DHA, i.e. 4*S*-hydroperoxy,17*S*-hydroxy-DHA, is transformed via an epoxide to resolvins D3 and D4 (illustrated). 17*R*- and 17*S*-hydroxy-DHA have anti-inflammatory properties of their own although they have generally been viewed simply as pathway markers and have been found in blood samples.

Interestingly, both 22:5(*n*-3) and 22:5(*n*-6) are also good substrates for 15-lipoxygenase, and the latter gives 17*S*-hydroxy-22:5(*n*-6) and 10,17*S*-dihydroxy-22:5(*n*-6) as the main products. Both of these (*n*-6) docosanooids are potent anti-inflammatory agents when administered either intravenously or orally.

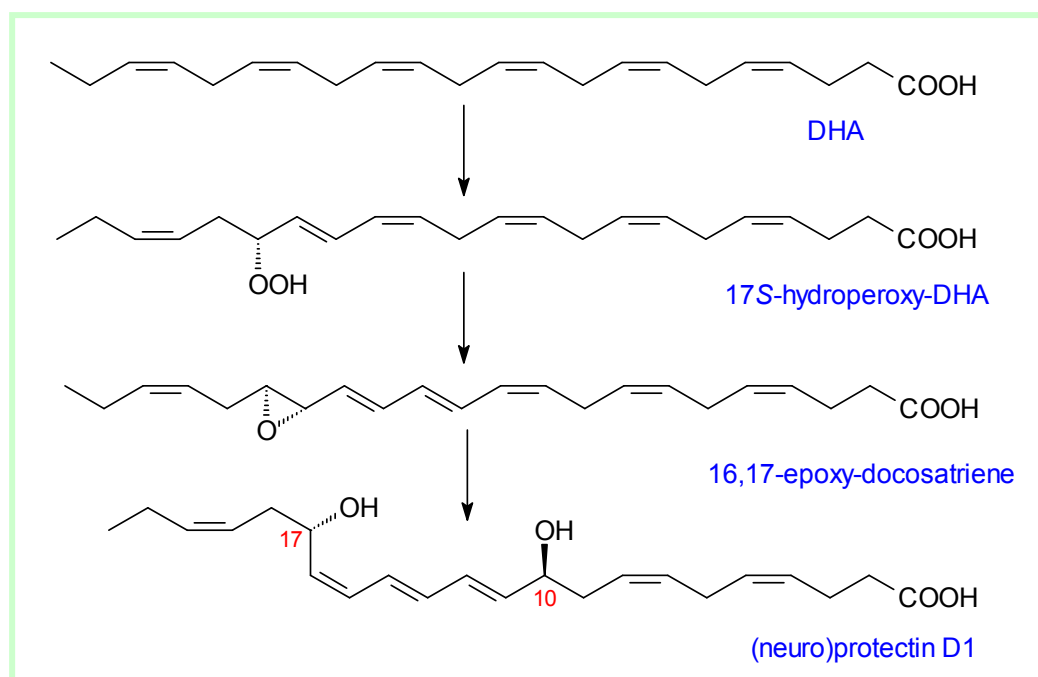


As with RvE1, RvD1 is de-activated by oxidation to 8- or 17-oxo-RvD as the first step in catabolism.

3. Protectins (Neuroprotectins)

In studies of resolvin formation in brain tissue in response to aspirin treatment, it was shown that new docosatrienes termed initially 'neuroprotectins' were produced. Like the leukotrienes, there are three double bonds in conjugation, hence the term 'triene', though there are six double bonds in total. As it is now recognized that the formation and actions of these docosanooids are not restricted to neuronal tissue, it has been suggested that the simpler term 'protectins' is preferable. For example, PD1 is present in murine inflammatory exudates and lung, in peripheral human blood and exhaled breath condensates, and in a wide range of cell types.

The biosynthetic pathway to neuroprotectin or protectin in brain tissue is illustrated below.



The lipoxygenase product 17*S*-hydroperoxy-DHA is converted first to a 16(17)-epoxide and then to the 10,17-dihydroxy docosatriene (10*R*,17*S*-dihydroxy-docosa-4*Z*,7*Z*,11*E*,13*E*,15*Z*,19*Z*-hexaenoic

acid), denoted as 10*R*,17*S*-DT or PD1 (or NPD1). Synthesis of NPD1 is induced as a response to oxidative stress and/or activation of neurotrophins, and again it appears that this highly stereospecific structure is essential for biological activity.

Further oxygenation can occur, and 17*S*-hydroperoxy-DHA can be oxidized at the terminal carbon atom by cytochrome P450 enzymes or it can react with 5-lipoxygenase to form two regioisomeric dihydroxy products, for example. An alternative single oxygenation is found in human macrophages and platelets in which a mediator termed **maresin 1** (7*S*,14*S*-dihydroxy-docosa-4*Z*,8*E*,10*E*,12*Z*,16*Z*,19*Z*-hexaenoic acid) is formed via the action of human 12-lipoxygenase. In addition, similar oxygenated compounds with anti-inflammatory properties are formed from 22:5(*n*-3) and 22:5(*n*-6) fatty acids.

4. Biological Activity

Acute inflammation in response to infection or tissue damage is usually characterized by heat, redness, swelling and pain at a simple observational level, and by oedema, accumulation of leukocytes, and then by accumulation of monocytes and macrophages at a cellular level. Leukotrienes (especially LTB₄) and prostaglandins (PGE₂ and PGD₂) derived from arachidonic acid are important in the early stages of the inflammatory process. As tissues return to health, resolvins and protectins, together with lipoxins and maresins, promote resolution of the inflammation through removal of the leukocytes together with cellular debris, ideally without leaving remnants of the host defences or of the invading microorganisms or other inflammatory initiators.

The resolvins and neuroprotectins are distinctive and highly stereospecific lipids, which are endogenous local mediators with strong anti-inflammatory effects in addition to some immunoregulatory activities at picomolar to nanomolar concentrations. They are part of the molecular mechanisms that contribute to removal of inflammatory cells and restoration of tissue integrity once the need for the inflammatory response is over, i.e. they actively assist in the resolution of inflammation, once thought to be a passive process. It is evident that the presence of aspirin uniquely facilitates this resolution. Thus, at local sites of inflammation, aspirin treatment enhances the conversion of the omega-3 fatty acids EPA and DHA to 18*R*-oxygenated products, i.e. resolvins of the E and D series, which carry potent anti-inflammatory signals. So far two receptors have been identified that mediate the activities of RvE1.

During inflammation, polymorphonuclear neutrophils are produced which have generally beneficial effects in countering disease, but in the longer term or if malfunctioning they may eventually cause trauma and tissue damage through infiltration into tissues. The resolvins, like the **lipoxins**, appear to have an important role in regulating and indeed inhibiting these harmful effects. In so doing they oppose the effects of some of the pro-inflammatory prostanoids. For example, nanomolar concentrations of resolvin E1 dramatically reduce dermal inflammation, peritonitis, dendritic cell migration and interleukin production. RvE1 blocks excessive platelet aggregation, and it also limits the effects of certain human pathogens by enhancing phagocytosis by polymorphonuclear leukocytes. Similarly, RvD2 has extremely potent regulatory actions on neutrophil trafficking in the picogram range *in vivo* by stimulating resolution and enhancing innate host defense mechanisms via a specific receptor.

The (neuro)protectins appear to operate in the same way as the resolvins in brain tissue. Thus, (N)PD1 has anti-inflammatory effects and protects retinal epithelial cells from apoptosis induced by oxidative stress. In addition, it has protective effects in animal models of stroke and of Alzheimer's disease. Amongst its activities in non-neuronal tissues, it promotes apoptosis of T cells and it has beneficial effects towards asthma in nanogram amounts. It is evident that such compounds and their metabolism have considerable potential for therapeutic intervention in acute inflammation or chronic inflammatory disease. They may also mitigate the affects of sepsis.

It is now well established that administration of lipoxins, resolvins and protectins *in vivo* in animal models can aid the process of recovery from inflammation without compromising host defences by causing immune suppression. It is evident that these lipids or synthetic analogues have considerable therapeutic potential in managing chronic inflammatory diseases, including arthritis, cardiovascular disease, asthma and even cancer. From a nutritional or health standpoint, it has been suggested that dietary supplements of the precursor *omega*-3 fatty acids, taken together with aspirin, may ameliorate the clinical symptoms of many inflammatory disorders by regulating the time course of resolution via the production of resolvins and protectins.

Recommended Reading

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