The Octadeca-9,12-dienyl Sulphate Anion; a New, Water-soluble Substrate for Lipoxygenase

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Summary Linoleyl sulphate, the title compound, is a substrate for lipoxygenase, and is water-soluble throughout the whole range of pH in which the enzyme shows activity.

LIPOXYGENASE (EC.1.99.2.1.) catalyses the oxidation of compounds containing the *cis,cis*-penta-1,4-diene system, forming the conjugated *cis,trans*–diene hydroperoxide.[1] The anion of linoleic acid (octadeca-9,12-dienoic acid) is the most commonly used substrate, forming the anion of 13-hydroperoxyoctadeca-9,11-dienoic acid on oxidation, but studies of the reaction rate with pH are hampered by the insolubility of linoleic acid below about pH 7.[2,3] Attempts to overcome this difficulty by the addition of organic solvents to the reaction medium lead to the appearance of new and spurious peaks in the pH–activity profile; and dispersing the linoleic acid by means of a non-ionic detergent appears only to delay the onset of insolubility to a somewhat lower pH.[4] Thus the true pH–rate profile of lipoxygenase can only be determined by employing a substrate which is water-soluble over all the pH range in which the enzyme is active.

I report the preparation of, and preliminary work with, such a substrate, potassium octadeca-9,12–dienyl sulphate, *i.e.* potassium linoleyl sulphate. Methyl linoleate was reduced to linoleyl alcohol (octadeca-9,12-dien-1-ol) by $LiAlH_4$. Sulphation was achieved in good yield by heating linoleyl alcohol (1 mole), sulphamic acid (1·5 moles), and pyridine (2 moles) at 95°, under nitrogen for 1 hr.[5] Methanol was then added, and the solution was filtered and made alkaline with K_2CO_3. The resulting suspension was evaporated to dryness, keeping the temperature below 60°. Extraction of the solid and recrystallisation from methanol gave light-brown plates, which were further purified by chromatography on silica. Elution with benzene (95 parts)–methanol (5 parts) gave white plates, m.p. 161°. These were shown to be pure by t.l.c., and the i.r. spectrum showed all the characteristics of an alkyl sulphate. Two moles of H_2 were consumed on catalytic hydrogenation, to give a compound identical to potassium octadecyl sulphate. Finally, the substance acted as a substrate for lipoxygenase, thus confirming the presence of a *cis,cis*-methylene-interrupted diene system.

Studies of substrate concentration and enzyme activity showed that Michaelis–Menten kinetics were followed between pH 7·0 and 9·0. As with the linoleate anion,[4] the value of the Michaelis constant, K_m, did not change over the region covering the critical micelle concentration of the substrate. At pH 8·0 and ionic strength 0·1, $K_m = 2{\cdot}0 \times 10^{-5}$M; this is similar to, and possibly somewhat lower than, values obtained for the linoleate anion.[4,6] This suggests that the degree of binding to lipoxygenase is independent of the nature of the terminal anionic group of the substrate. A study of the effectiveness of linoleylamine (1-aminooctadeca-9,12-diene) as a substrate is now in progress.

Its rapid autoxidation makes quantitative studies difficult, and although it appears to act as a substrate for lipoxidase, it is not possible at present to say whether an anionic or a cationic substrate is more effectively bound to the enzyme.

The relationship between pH and V_{max} has been determined between pH 6·0 and 10·0, using linoleyl sulphate in buffers of ionic strength 0·1 (Figure 1). A characteristic

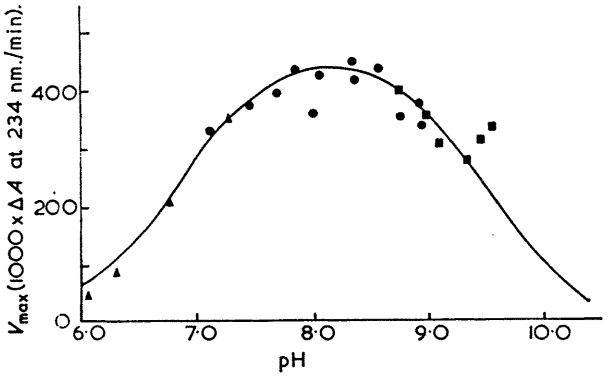

FIGURE 1. *pH–Activity profile of lipoxygenase, using linoleyl sulphate as substrate. Comparison of experimental data with computed curve. Buffers used were,* 0·1M-2-(N-*morpholino)ethanesulphonic acid (triangles),* 0·1M-*Tris (circles), and* 0·1M-*glycine (squares).* V_{max}, *the maximum activity, is expressed as* 1000 × *absorbance change at* 234 *nm. in standard assay. See ref.* 4 *for details.*

"bell-shaped" curve was followed between pH 6·0 and 9·5, with pH_{max} = 8·15 (pH_{max} = 7·9 for the linoleate anion, under similar conditions[4]), but turned upward between pH 9·5 and 10·0. The computed curve shown in Figure 1 was constructed from the experimental data, using the method of Lamden, Mathias, and Rabin.[7] The values obtained for the dissociation constants of the ionisable groups on the enzyme participating in the catalysis are given in the Table,

TABLE

Values obtained from work in buffers of ionic strength 0·1

Substrate	pH_{max}	pK_1	pK_2
Linoleate[a]	7·90	7·2	8·6
Linoleyl sulphate	8·15	6·8	9·4

[a] Values taken from ref. 4.

together with those from a similar system using the linoleate ion as the substrate.

A plot of pH against the Michaelis constant also gave the characteristic "inverted-bell" shape (Figure 2), of an enzyme

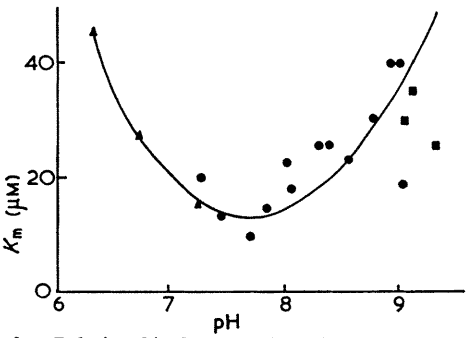

FIGURE 2. *Relationship between the Michaelis Constant* (K_m) *of lipoxygenase and pH. Buffers used were,* 0·1M-2-(N-*morpholino-ethanesulphonic acid (triangles),* 0·1M-*Tris (circles), and* 0·1M-*glycine (squares).*

with two ionisable groups necessary for activity, one in the acid- and one in the base-form. Some evidence exists to suggest that a histidine residue is one of these,[8] and the pK_1 of 6·8 is consistent with this; thiols, too, have been implicated in the catalysis,[9,10] and pK_2 may well correspond to the ionisation of such a group. The evidence from this work thus shows that, when the difficulties due to substrate insolubility are eliminated, lipoxygenase exhibits quite normal pH–activity relationships.

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