C_{18} unsaturated fatty acid hydrogenation patterns of some rumen bacteria and their ability to hydrolyse exogenous phospholipid

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1. A number of rumen bacteria isolated because of their ability to deacylate phosphatidylcholine, were found, in addition, to hydrogenate polyunsaturated fatty acids.

2. The most active lipolytic organisms had an unusual pattern of hydrogenation of dietary fatty acids in that α-linolenic acid was hydrogenated only as far as *trans*-11,*cis*-15-octadecadienoic acid.

It is now well known that the complex plant lipids present in the diet of ruminants are hydrolysed in the rumen and the polyunsaturated fatty acids released are hydrogenated by micro-organisms, mainly to stearic acid with a variable amount of *trans*-monoenoic acids (Garton, 1964; Dawson & Kemp, 1970). This process is thought to account, at least partially, for the high content of saturated fat in ruminant food products. A number of workers have isolated rumen bacterial species which can hydrogenate unsaturated fatty acids. Two distinct types of bacteria have been recognized: those which can convert linolenic and linoleic acids to *trans*-11-octadecenoic acid, but which are without action on oleic acid (Kepler, Hirons, McNiell & Tove, 1966; Dawson & Kemp, 1970; Kemp, White & Lander, 1975), and those which can hydrogenate oleic and linoleic acids to stearic acid, and α-linolenic acid to *cis*- and *trans*-15-octadecenoic acids (White, Kemp & Dawson, 1970; Kemp *et al.* 1975).

Recently in this laboratory we have isolated a number of bacterial species from the rumen which possessed appreciable lipolytic activity towards exogenous ^{32}P-labelled phosphatidylcholine substrate (Hazlewood & Dawson, 1975). Results of tests using ^{14}C-labelled fatty acids have indicated that those organisms which possessed significant lipolytic activity towards phosphatidylcholine were hydrogenators of polyunsaturated fatty acids. In addition those organisms with the highest lipolytic activity had a distribution of hydrogenation end-products different to those previously recognized.

METHODS

Lipolytic organisms were isolated by the techniques described by Hazlewood & Dawson (1975) using the selective linseed oil-containing medium of Hobson & Mann (1971). Their ability to release fatty acids from a lipid substrate was monitored by measuring the production of water-soluble radioactivity from [^{32}P]phosphatidylcholine added to cultures (Hazlewood & Dawson, 1975). Other hydrogenating bacteria

were isolated as described by Kemp *et al.* (1975). Organisms were maintained on agar slopes containing glucose (5 g/l) and subcultured every month. To study their hydrogenating ability, bacteria were grown in a non-selective rumen fluid-containing liquid medium (Bryant & Robinson, 1961), slightly modified to include yeast extract (1 g/l) and agar (0·5 g/l). 1-^{14}C-labelled oleic, linoleic or α-linoleic acids (0·5 μCi, 10 μg) were added to 5 ml medium before autoclaving. Inoculation and incubation for 48 h at 39 ° were both carried out under a gas phase of pure carbon dioxide, using open-tube techniques (Latham & Sharpe, 1971).

Analysis of the hydrogenation products, including determination of the positions of the double bonds by oxidation using permanganate–periodate was carried out by a combination of radio–gas–liquid chromatography, and thin-layer chromatography on silver nitrate-impregnated silica gel (Kemp & Dawson, 1968).

RESULTS AND DISCUSSION

The results of these studies are given in Table 1, in which a series of organisms originally isolated for their ability to hydrolyse phosphatidylcholine (Hazlewood & Dawson, 1975) are compared with other organisms which were retained after isolation because of their hydrogenation activity (Kemp *et al.* 1975). The organisms which had an ability to deacylate phosphatidylcholine had, without exception, a capacity for hydrogenation. On the other hand, not all hydrogenating bacteria were lipolytic, as was found with the organisms P2/2C and 2/7/2 (Table 1).

There were marked differences in the hydrogenation reactions carried out by the different bacteria, and three distinct patterns of hydrogenation were apparent. The most active lipolytic organisms (LM8/1B, LM8/1A, R8/3) could not hydrogenate oleic acid; linoleic acid was converted exclusively to *trans*-11-octadecenoic acid, and α-linoleic acid to *trans*-11, *cis*-15-octadecadienoic acid. This pattern was also found in a non-lipolytic organism 2/7/2. These bacteria (group 1) presumably possess the Δ12-*cis*, Δ11-*trans*-isomerase (*EC* 5.2.1.5) described by Kepler, Tucker & Tove (1971), which can convert linoleic acid and α-linoleic acid to *cis*-9, *trans*-11-octadecadienoic acid and *cis*-9, *trans*-11, *cis*-15-octadecatrienoic acid respectively, and can then subsequently hydrogenate the *cis*-9 bond in the polyenoic acids, but they are incapable of the isomerization and hydrogenation of the *cis*-15 bond of linoleic acid or the *cis*-9 bond of oleic acid. A second group of organisms (R7/5, 2/9/1, EC7/2) which were less actively lipolytic, were again unable to hydrogenate oleic acid, while both linoleic and α-linolenic acid were converted to a variety of *trans*- and *cis*-positional isomers of octadecenoic acid of which *trans*-11 predominated. This group therefore had the same isomerase properties as the bacteria of group 1 except that in addition they had the capacity to either isomerize the *cis*-15 double bond to a more suitable position for hydrogenation, or to hydrogenate it directly (e.g. linolenic acid was converted to *trans*-11-octadecenoic acid). In a third group of bacterial isolates with marginal or no lipolytic activity (P2/2C, R8/5, T344) both oleic and linoleic acids could be hydrogenated to stearic acid and α-linolenic acid to *cis*- and *trans*-isomers of 15-octadecenoic acid. With all substrates, the minor products often included a number

Table 1. *Lipolytic activity and ability of a series of rumen bacterial isolates to hydrogenate* 1-^{14}*C-labelled oleic, linoleic and linolenic acids*

(Values in parentheses are the percentages of the total radioactivity recovered in each compound)

Substrates ...	Organism		Lipolytic activity towards [^{32}P]phosphatidylcholine (% decomposition of substrate in 24 h)	Oleic acid (18:1, *cis*-9)	Linoleic acid (18:2, *cis*-9, *cis*-12)	Linolenic acid (18:3, *cis*-9, *cis*-12, *cis*-15)
	Group no.	Identity		Main metabolic products or unchanged substrate recovered		
	1	LM8/1B, Gram-negative rod	100	18:1, *cis*-9 (100)	18:1, *trans*-11 (100)	18:2, *trans*-11, *cis*-15 (100)
		LM8/1A, Gram-negative rod	99	18:1, *cis*-9 (100)	18:1, *trans*-11 (100)	18:2, *trans*-11, *cis*-15 (100)
		R8/3, Gram-negative rod	99	18:1, *cis*-9 (100)	18:1, *trans*-11 (96)	18:2, *trans*-11, *cis*-15 (100)
		2/7/2	0	18:1, *cis*-9 (100)	18:1, *trans*-11 (75) 18:2, *cis*-9, *cis*-12 (25)	18:2, *trans*-11, *cis*-15 (100)
	2	R7/5, Gram-negative rod	14	18:1, *cis*-9 (100)	18:1, *trans*:*cis* ratio 1:2 (95) *trans*-11 (14) *trans*-10 (11) *trans*-12 (8) *cis*-11 (62) *cis*-10 (5) 18:2 (trace)	18:1, *trans*:*cis* ratio 1:1 (100) *trans*-11 (22) *trans*-10 (15) *trans*-12 (6) *cis*-11 (44) *cis*-10 (5)
		EC7/2, Gram-negative rod	17	18:1, *cis*-9 (100)	18:1, *trans*-11 (100)	18:2, *trans*-11, *cis*-15 (70) 18:1, *trans*-11 (30)
		2/9/1, Gram-negative vibrio	8	18:1, *cis*-9 (100)	18:1, *trans*:*cis* ratio 2·5:1 (100) *trans*-11 (46) *trans*-12 (12) *trans*-10 (15) *cis*-11 (25) *cis*-12 (5)	18:1, *trans*:*cis* ratio 2:1 (95) *trans*-11 (41) *trans*-12 (11) *trans*-10 (14) *cis*-11 (29) *cis*-12 (4) 18:2 (trace)
	3	P2/2C, *Fusocillus babrahamensis*	0	18:0 (70) 18-OH (20)	18:0 (70) 18:1, *trans*-11 (20) 18:2, *cis*-9, *cis*-12 (10)	18:1, *cis*-15 (85) *trans*-15 (15)
		R8/5, Gram-negative rod	4	18:0 (40) 18:1, *cis*-9 (60)	18:0 (40) 18:1, *trans*-11 (50)	18:2, *trans*-11, *cis*-15 (25) 18:1, *cis*-15 (50) *trans*-15 (25)
		T344, *Fusocillus* sp.	2	18:0 (90) 18:1, *trans*-11 (5) 18:1, *cis*-9 (5)	18:0 (75) 18:1, *trans*-11 (15) plus other *trans*-isomers	18:1, *cis*-15 (85) *trans*-15 (10) *trans*-14 (5)

Table 2. *Isomerization and hydrogenation activities of a series of rumen bacterial isolates towards double bonds in* 1-^{14}*C-labelled oleic, linoleic and linolenic acids*

Type of double bond	...	*Cis*-9		*Cis*-15
Group no.*	In oleic acid	After isomerization of linoleic and α-linolenic acids	In *trans*-11,*cis*-15 octadecadienoic acid	
1	−	+	−	
2	−	+	+	
3	+	+	−	

+, Positive; −, negative.
* For details, see Table 1.

of positional isomers of octadecenoic acid. Clearly therefore these group 3 organisms possess, unlike the previous two groups, an ability to isomerize and hydrogenate non-conjugated double bonds (e.g. oleic acid, *trans*-11-octadecenoic acid), while in similar manner to group 1, and unlike group 2 organisms, they cannot positionally isomerize and hydrogenate double bonds in the C-15 position of C_{18} fatty acids (linolenic acid is converted to *cis*- and *trans*-15-octadecenoic acid). An octadecenoic acid isomerase with a high specificity (*cis*-9 → *trans*-10) has recently been described in *Pseudomonas sp.* NRRL B 3266 (Mortimer & Niehaus, 1974).

The highly organized isomerization and hydrogenation patterns found in these different bacterial groups (summarized in Table 2) could suggest that such organisms may use hydrogenation processes for specific purposes which are species dependent rather than for a more general biochemical process, such as disposal of hydrogen atoms. It seems likely that in the mixed rumen flora, group 2 bacteria play an important part in the metabolism of linolenic acid, the most common dietary fatty acid of ruminants on pasture, since they produce *trans*-11-octadecenoic acid which is readily hydrogenated in the rumen. Thus stearic acid, rather than 15-octadecenoic acid, is the main end-point of hydrogenation although the latter acid has been detected in small quantities in ruminant digesta passing to the duodenum (Bickerstaffe, Noakes, & Annison, 1972).

It should be emphasized that the properties of lipolysis of phosphatidylcholine and hydrogenation are found individually in only a small proportion of isolated rumen bacteria. Thus their common joint occurrence in a number of bacterial species suggests a connexion between the two phenomena rather than a chance happening. The ability to carry out both processes would be an advantage to any organism which had a physiological need for hydrogenation, since hydrogenation of unsaturated C_{18} dietary fatty acids esterified in the complex lipids (galactolipids, phospholipids in pasture, triglycerides in concentrates) cannot take place until the free carboxylic acid group has been exposed by lipolysis (Kepler *et al.* 1971; Dawson, Hemington, Grime, Lander & Kemp, 1974). Thus lipolysis and hydrogenation could occur in a single organism without the necessity for transfer of a water-insoluble fatty acid substrate from one bacterial species to another. However, it would seem that this transfer may take place since, up to now, it has not been possible to isolate a single bacterial species from

the rumen which can carry through the conversion of complex lipids to stearic acid, as occurs in the rumen. The alternative would be that in the rumen certain hydrogenases are truly extracellular and can diffuse to the substrate (Harfoot, Noble & Moore, 1973). However up to now there have been no reports of a soluble fatty acid hydrogenating system produced by rumen bacteria grown in isolation.

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