The Effect of Surface-active Agents on Pancreatic Lipase

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It has been known for a considerable time, e.g. Rachford (1891), that the addition of bile salts increases the rate of hydrolysis of fat by pancreatic lipase. Bile salts could speed up the rate of hydrolysis by activating the enzyme molecule, but since they are surface-active agents and therefore emulsifying agents it is clear that their activating effect could be simply due to an emulsification of the fatty substrate. A dual role is also possible, i.e. a combination of activation and emulsification.

Evidence available in the literature describing the mode of action of the bile salts does not give conclusive support for either the emulsification or the activation hypothesis. Thus, Willstätter, Waldschmidt-Leitz & Memmen (1923) and Willstätter & Memmen (1924) studied the activation of lipase by bile salt and observed that some crude lipase preparations could not be activated. Further, addition of ovalbumin and Ca^{2+} increased the rate of fat hydrolysis so that further activation by bile salts was sometimes impossible. Bile salts were found to activate lipase in an alkaline medium but to inhibit it in an acid medium. Weinstein & Wynne (1936) found that bile salts, at pH 7.2 and in concentrations $0.5\text{--}4 \times 10^{-4}\text{M}$, had no effect on the action of lipase. Glick & King (1932) found that the hydrolysis of tributyrin at pH 7.0 was inhibited by sodium taurocholate and sodium glycocholate but that the hydrolysis of triolein was accelerated. Holwerda (1938) concluded that sodium glycocholate promoted adsorption of the lipase on the fat. Krahling & Weber (1938) stated that bile salts affect the rate of hydrolysis of water-soluble esters by pancreatic lipase without affecting the enzyme-substrate affinity.

It was considered that the use of some synthetic detergents which aid fat emulsification would help to establish the significance of the role of emulsification in the digestion of fats by lipase. The emulsification theory would, for example, be strongly supported if it were found that the synthetic detergents, which are very unlikely to be enzyme activators, stimulate lipase action. A secondary object of the investigation was to establish whether these synthetic detergents, which are representative of those widely used industrially and domestically, had any marked inhibitory effect on the action of

digestive lipase. A number of synthetic detergents have now been examined; a few preliminary results were recorded previously (Wills, 1954).

MATERIALS

Lipase

An extract of hog pancreas prepared as previously described (Wills, 1954) was used for most experiments. Ox pancreas extract was used for a few experiments.

Detergents

Detergents were obtained from the following sources:

Anionic detergents. Teepol XL (sodium salt of a secondary alkyl sulphate), Tergitol 7 (sodium salt of a secondary C_{17} alkyl sulphate), Irgalon BT (sodium salt of ethylenediaminetetraacetic acid) and Manoxol OT (sodium salt of the dioctyl ester of sulphosuccinic acid) from British Drug Houses Ltd.; sodium dodecyl sulphate (SDS) from L. Light and Co. Ltd.; and Perminal COL (sodium salt of a highly sulphonated oil with fatty alcohol-polyethylene oxide condensate), Calsolene oil HS (a highly sulphonated oil), Dispersol LN (sodium naphthalenesulphonate), Lissapol C paste (sodium oleyl sulphate and water), Lissapol LS paste (sodium oleyl *p*-anisidinesulphonate and water) were gifts from Imperial Chemical Industries (Pharmaceuticals) Ltd.

Liquidol Roche (sodium polyanetholsulphonate) was obtained from Roche Products Ltd.

Cationic detergents. Hexadecylpyridinium bromide and hexadecyldimethylbenzylammonium chloride were obtained from L. Light and Co. Ltd., Gemex G (constitution not available) from British Drug Houses Ltd., and Fixanol VR (tetradecylpyridinium bromide) and Lissolamine A 50 paste (hexadecyltrimethylammonium bromide and water) were gifts from Imperial Chemical Industries (Pharmaceuticals) Ltd.

Non-ionic detergents. Stergene (an alkylaryl polyethoxyethanol) was obtained from Domestos Ltd., and Lubrol MO (a fatty alcohol-ethylene oxide condensate), Lubrol W (hexadecanol-polyoxyethylene condensate), Lissapol NX (polyoxyethylene condensate), Crill No. 6 (polyoxyethylene sorbitan monolaurate) were gifts from Imperial Chemical Industries (Pharmaceuticals) Ltd. Digitonin was obtained from British Drug Houses Ltd.

METHODS

Lipase activity

Lipase action was estimated by two methods:

(1) A manometric method at 37° and pH 7.4 was used and was essentially that previously described (Wills, 1954)

except that in the present series of experiments the triolein (0.3 ml.) was usually placed in the main compartment of the manometer and the enzyme solution (0.3 ml.) added from the side bulb.

(2) The method of Balls, Matlack & Tucker (1937) was used with slight modifications. In a typical experiment triolein (1 ml.), M ammonium chloride buffer (pH 8.5, 2.0 ml.) and water (26 ml.) were placed in a conical flask which was corked and shaken at 37° for 10 min. The experiment was started by adding lipase solution (1 ml.), and 5 ml. samples were removed immediately after addition of the enzyme and at 15 min. intervals for 60 min. The subsequent procedure was as described by Balls *et al.* (1937). In some experiments triacetin or tributyrin was used as substrate. In this method the pH of the mixture is kept approximately constant by adding ammonia at intervals to bring to pH 8.5 (as determined by the glass electrode).

The reaction mixture was mechanically shaken 100 times per minute with an amplitude of 4 cm. in both methods. Conical Warburg flasks of approx. 20 ml. capacity were used for method 1 and 100 ml. conical flasks were used for method 2.

RESULTS

Anionic detergents

The effect of 11 different anionic detergents (Permal COL, Calsolene oil HS, Dispersol LN, Teepol XL, Lissapol C paste, Lissapol LS paste, SDS, Tergitol 7, Liquoid Roche, Irgalon BT and Manoxol OT) on the hydrolysis of triolein by lipase was examined. Each detergent was used in concentrations 0.03–2.0% (w/v) and in each experiment was shaken with buffer and triolein for 10 min. before the reaction was started by addition of lipase. In practically every case when the detergent concentration was greater than 0.05% inhibition of lipase action was produced and complete inhibition occurred when high concentrations were used; for example in concentrations of 0.15 and 0.5% (w/v) respectively, SDS and Teepol XL caused 100% inhibition. In general, inhibition was more powerful when using the titration method than when using the manometric method. Liquoid Roche was the only member of this group which caused a stimulation of the rate of hydrolysis, 0.5% (w/v) increasing the rate about twofold. Permal COL and Calsolene oil HS were hydrolysed by lipase under the conditions of the methods used, particularly quickly in the manometric method.

The hydrolysis of fats by pancreatic lipase is accelerated by Ca^{2+} (Bamann & Laeverenz, 1934; Schönheyder & Volquartz, 1945), and in the present series of experiments 0.01 M- Ca^{2+} was found to be an optimum concentration and to increase the hydrolysis rate by the factor 7.1. The effect of Manoxol (1%, w/v), Irgalon BT (1%, w/v) and SDS (0.01 M) on lipase was examined in presence of 0.01 M- Ca^{2+} . Each detergent caused powerful inhibition (90–100%) of the enzyme.

For comparison with the effect of detergents used in this work sodium taurocholate and sodium deoxycholate were tested on lipase in concentrations which varied from 0.1 to 5.0% (w/v). Providing the pH was 7.0 or more alkaline the hydrolysis of triolein was accelerated, the extent of the stimulation increasing as the concentration of bile salt was increased. The anionic detergents, Irgalon BT (1.0%, w/v), Manoxol (0.1%, w/v), Calsolene oil HS (1.0%, w/v), Permal C (1.0%, w/v) and SDS (0.3%, w/v) were tested on lipase in presence of 1% (w/v) sodium taurocholate. This concentration of bile salt used alone powerfully stimulated the rate of triolein hydrolysis but only a little change in this rate was caused by addition of the detergent, although some of these detergents, when used alone, inhibited the hydrolysis.

Schulman & Cockbain (1940) observed that the emulsification of oil by SDS was markedly improved by dissolving cholesterol (10 mg./ml.) in the oil. A solution of cholesterol in triolein (10 mg. cholesterol/ml.), was used as substrate for lipase and the rate of hydrolysis was found to be increased by the factor 2.75 as compared with the rate for untreated triolein. Very good emulsification of cholesterol-treated triolein was obtained when the optimum quantity of SDS (1.75 mg./ml. aqueous phase, as suggested by Schulman & Cockbain, 1940) was used, but despite the adequate emulsification the rate of hydrolysis was less than that of cholesterol-treated triolein in absence of SDS. The SDS caused 31% inhibition in this case, although the same concentration of detergent completely inhibited the hydrolysis of untreated triolein.

Triacetin as substrate. When neutralized triacetin was used as substrate, 0.001 M SDS gave 58–92% inhibition over the pH range 6.2–7.6. Teepol (3%, w/v) completely inhibited the hydrolysis but 0.3% (w/v) Liquoid Roche did not affect it.

Tributyrin as substrate. Although SDS did not stimulate the action of lipase on triacetin or triolein under any conditions used, certain concentrations of this detergent greatly accelerated the enzymic hydrolysis of tributyrin. Strong solutions of SDS (0.01 M) inhibited the hydrolysis and 0.001 M SDS, which increased the rate tenfold, was found to be the concentration for maximum stimulation. In the absence of enzyme, SDS (0.001 M) had no hydrolytic action. Addition of Ca^{2+} (0.01 M), which alone stimulated the rate of hydrolysis by the factor 2.4, in presence of 0.001 M SDS gave a rate of hydrolysis nearly equal to that of the control (no added Ca^{2+} or SDS), i.e. the stimulating effects of both were abolished. This may be due to the precipitation of SDS as the calcium salt. Manoxol (0.1%, w/v) was also tested and found to increase the rate of hydrolysis of tributyrin by the factor 4.0.

The effect of cationic detergents

Five cationic detergents in concentrations which varied from 0.001 to 2.0% (w/v) were tested for their effects on lipase action. All these cationic detergents, hexadecylpyridinium bromide, Fixanol VR, Lissolamine A50 paste, hexadecyldimethylbenzylammonium chloride and Gemex G, were found under certain conditions to increase the rate of hydrolysis of triolein. In general, stimulation was greater when using the manometric method than when using the titration method. The concentration of detergent which gave the most powerful stimulation was not the same for each method; thus, when using the titration method, maximum stimulation (2.5 times the control rate) for hexadecylpyridinium bromide was obtained in 0.04% (w/v) solution but using the manometric method for maximum

quantity of triolein and the detergent concentration were both varied. It will be seen that, as more triolein is used, more detergent is necessary to give the maximum rate and that this maximum appears to be when the ratio by wt. hexadecylpyridinium bromide/triolein is approx. 0.01, whatever the quantity of triolein. Hexadecylpyridinium bromide (0.01M) did not hydrolyse triolein to any measurable extent in absence of enzyme.

Table 1. *The effect of hexadecylpyridinium bromide on triolein hydrolysis when the detergent/triolein ratio is varied*

The titration method was used for all the experiments. The rate of acid liberation expressed as m-equiv./hr. was 0.46 in control experiment (1), 0.37 in control (2) and 0.32 in control (3).

Fig. 1. The effect of hexadecylpyridinium bromide on the hydrolysis of triolein by lipase. The rate of hydrolysis is expressed in arbitrary units, taking that of the control in absence of detergent as 100. The rate of acid liberation in the control experiment was 0.32 m-equiv./hr.

stimulation (11.0 times the control) was obtained at 0.15% (w/v). The difference in concentrations for maximum stimulation is considered to be due mainly to the different oil/aqueous phase ratio used in the two methods, it being 1/10 (v/v) in the manometric method and 1/30 (v/v) in the titration method. Results using various concentrations of hexadecylpyridinium bromide are summarized in Fig. 1, which shows that a certain concentration of detergent gives powerful stimulation but that more concentrated solutions cause inhibition. Emulsification was observed to be most effective, judged visually, in those experiments in which powerful stimulation was produced. The increase of hydrolysis rate was found to depend essentially on the detergent/oil ratio, as shown in Table 1 where the

Detergent concn. (10 ⁻³ M)	Ratio triolein/detergent (by wt.)	Rate of hydrolysis (% of control without detergent)
(1) Using 4.5 g. triolein and 15 ml. aqueous phase		
1.33	60	195
0.67	119	195
0.27	298	145
0.13	596	47
(2) Using 1.83 g. triolein and 20 ml. aqueous phase		
1.0	24	28
0.5	48	50
0.2	119	178
0.1	238	103
(3) Using 0.915 g. triolein and 30 ml. aqueous phase		
1.0	7.9	30
0.2	40	90
0.1	80	250
0.05	158	190
0.033	238	127
0.02	396	110

In all experiments so far described triolein was mixed with detergent and buffer and the hydrolysis started by addition of enzyme. If, however, the order of addition was altered so that the detergent had prior contact with the enzyme and the experiment was started by adding triolein, 0.001M hexadecylpyridinium bromide, which strongly activated if first mixed with triolein, now produced marked inhibition (Fig. 2).

Cationic detergents in concentrations which increased the rate of triolein hydrolysis in absence of Ca²⁺ were tested in the presence of 0.01M-Ca²⁺. Gemex G and Fixanol VR were the only detergents which stimulated the rate of hydrolysis, Gemex G increasing the rate to 3.3 times the control rate. Lissolamine A50 (0.05%, w/v) and 0.001M hexadecylpyridinium bromide strongly inhibited, although these detergents stimulated at these concentrations in absence of Ca²⁺.

The five cationic detergents were tested on lipase in presence of 0.3, 0.5 and 1.0% (w/v) sodium taurocholate. All detergents were used in concentrations which, when used alone, increased the rate of triolein hydrolysis, but in most cases the same concentration of detergent used with the bile salt gave a rate of hydrolysis which was less than that when using the bile salt alone. In those cases where the rate of hydrolysis was increased, the resulting

Fig. 2. The effect of 0.001 M hexadecylpyridinium bromide on hydrolysis of triolein by lipase when the order of addition of the reactants is varied. A, hexadecylpyridinium bromide added to triolein and the experiment started by addition of enzyme after 15 min.; B, triolein and enzyme without detergent; C, hexadecylpyridinium bromide added to the enzyme and the experiment started by addition of triolein after 15 min.

rate was always considerably less than the sum of the rates of hydrolysis produced by the addition of either the bile salt alone or detergent alone. For example, in one experiment sodium taurocholate alone increased the hydrolysis rate 10.4 times whilst Lissolamine A 50 increased the rate 6.0 times; if both detergent and bile salt were added together the control rate was increased 10.0 times, i.e. the effect was very little different from that of the bile salt alone. If, however, as has been previously noted for hexadecylpyridinium bromide, the detergent had prior contact with the enzyme, complete inhibition was produced even though bile salt was present.

Hexadecylpyridinium bromide (0.01 and 0.005 M) caused strong inhibition of the hydrolysis of triolein

containing cholesterol (10 mg. cholesterol/ml. triolein).

Triacetin as substrate. Hexadecylpyridinium bromide used in concentrations which increased the hydrolysis rate of triolein was tested on the hydrolysis of triacetin. This detergent inhibited the enzyme action at all concentrations tested. Thus, 0.004 M hexadecylpyridinium bromide caused 93%; 0.002 M, 79%; 0.001 M, 45%; and 0.0003 M, 23% inhibition.

Tributylin as substrate. The effect of hexadecylpyridinium bromide on tributyrin hydrolysis was examined using the titration method. As with triolein hydrolysis, a critical concentration of detergent, in this case about 0.0005 M, was found to give optimum hydrolysis of tributyrin.

The effect of non-ionic detergents

Six non-ionic detergents (Lubrol MO, Lubrol W, Lissapol NX, Crill No. 6, digitonin and Stergene) were tested in concentrations ranging from 0.01 to 5.0% (w/v). These non-ionic detergents inhibited triolein hydrolysis by lipase when used in high concentrations; thus for example 1.0% (w/v) digitonin caused 60% inhibition and 0.1% Lissapol NX 80% inhibition; all these detergents had no effect in solutions less than 0.02% (w/v). Of the detergents tested, only Lubrol MO, which is insoluble in water and was used as a suspension, was found to increase the rate of hydrolysis, and this only to a small extent.

Stergene and digitonin were tested in presence of 0.01 M-Ca²⁺. Stergene (1%, v/v) caused 8% inhibition of triolein hydrolysis in presence of 0.01 M-Ca²⁺ whilst digitonin (1%, w/v) caused 60% inhibition under these conditions when tested manometrically.

Stergene (10%, v/v) completely inhibited the hydrolysis in presence of 0.3% (w/v) sodium taurocholate, but 1% (v/v) Stergene had no effect under these conditions.

Triacetin as substrate. Stergene (10%, v/v) caused 67% inhibition of triacetin hydrolysis.

Tributylin as substrate. Lissapol NX was found to inhibit tributyrin hydrolysis: 1% (w/v) produced 100% inhibition; 0.5% (w/v), 94%; 0.1% (w/v), 70%; 0.025% (w/v), 46%. These results were thus comparable with those obtained using triolein.

Ox pancreas enzyme

The lipase extracted from ox pancreas was tested in some experiments using SDS, hexadecylpyridinium bromide and Lissolamine A 50. The activity of the preparation was less than that of the hog enzyme but in general the results using these detergents agreed closely with those previously recorded in this paper.

DISCUSSION

Most of the anionic detergents tested were found to inhibit lipase action, Liquoid Roche being the only anionic detergent found to produce an increase in the rate of triolein hydrolysis, and this increase was very small. When sodium taurocholate (1%, w/v) was added, however, the anionic detergents had little or no effect on the rate of triolein hydrolysis, the enhanced rate of hydrolysis due to presence of bile salt remaining practically unchanged. The detergents used, e.g. 0.01M SDS, powerfully inhibited the enzyme in absence of bile salt so that the bile salts must have exerted some protective action.

Addition of cholesterol to triolein strongly stimulates the rate at which this fat is hydrolysed by pancreatic lipase. This fact suggests a possible role for the cholesterol in bile, which is usually stated to contain 0.4% (w/v) cholesterol, so that 1 ml. bile will be capable of activating 40 ml. triolein under the conditions used in the present investigation. The addition of an aqueous solution of SDS to a solution of cholesterol in triolein produced a very good stable emulsion but the rate of hydrolysis of this emulsion was low and less than the control using an unemulsified oil. It is clear therefore that good emulsification alone is insufficient to give a rapid rate of hydrolysis. Possible stimulation of the rate of hydrolysis by the emulsification is apparently overcome by the powerful inhibitory effect of SDS on the enzyme.

Results of experiments using the non-ionic detergents are in agreement with those found for the Tweens 20, 60 and 80 (Atlas Powder Co.) by Minard (1953). Lubrol MO in high concentration was the only member of this group which would produce any increase in the rate of hydrolysis under any conditions tested.

All cationic detergents used in this series of experiments when tested under suitable conditions produced an increase in the rate of hydrolysis of triolein. For each detergent a critical concentration was found which produced maximum stimulation, but when more concentrated solutions were used inhibition usually occurred (Fig. 1). If excess detergent is added, although emulsification may still appear to be good as judged by eye, there will almost certainly be surplus detergent in the aqueous phase, which will cause inhibition of the enzyme. At the critical concentration the detergent and oil are probably closely bound in globules in the emulsion and the detergent is then prevented from inhibiting the enzyme. That these detergents will inhibit lipase is clearly shown in Fig. 2 where the normal order of addition is reversed. In this experiment hexadecylpyridinium bromide, at a concentration which increased the hydrolysis rate when added to

the triolein, inhibited when added first to the enzyme. Emulsification does appear, however, to be an important factor in increasing the velocity of hydrolysis, since in general it was observed that the hydrolysis rate was greatest when the emulsification was best, provided the least quantity of detergent which would produce this emulsification was used. At their critical concentrations the cationic detergents tested are much more effective weight for weight than the natural bile salts tested. Thus, for example, 1% (w/v) sodium deoxycholate increased the rate of hydrolysis of triolein by the factor 7.5, whilst under the same conditions 0.05% (w/v) hexadecylpyridinium bromide increased the velocity by 14.3; thus the detergent is nearly 40 times more effective than the bile salt. The fact that the positively charged cationic detergents increased the rate of triolein hydrolysis so effectively is surprising in view of the fact that the natural activators, the bile salts, are anionic and negatively charged. Although in dilute solutions the cationic detergents tested increase the rate of hydrolysis, they inhibit in concentrated solution, a property that is not shared by the bile salts, which do not inhibit at any concentration up to the maximum tested (5%, w/v), in neutral or in alkaline solution.

Experiments using cationic detergents and bile salts show that, in the main, these detergents cannot stimulate the hydrolysis over and above that produced by 1% (w/v) sodium taurocholate. In no case were the increase of rate due to bile salt alone and that due to detergent alone additive, and in several cases a mixture of bile salt and detergent in optimum concentration gave a rate which was less than that of the bile salt used alone. These results, it is considered, support the view that the main function of bile salts is to emulsify the triolein, since if they were true enzyme activators their addition to the oil, already well emulsified by detergent and giving a rapid hydrolysis with untreated enzyme, should give a much more rapid rate if the bile salt was also added. If, however, the role of the bile salt is primarily one of emulsification, then the results may be easily explained on the view that emulsification is adequate, using either detergent or bile salt, and addition of the two together would not be expected to produce a rate greater than that using bile salt alone.

Hexadecylpyridinium bromide inhibited triacetin hydrolysis in a concentration which increased the rate of triolein hydrolysis. Since under these conditions the detergent is not a true enzyme activator and not bound up in an emulsion, it is free to inhibit the lipase.

The fact that many of the detergents used were commercial samples and not pure substances did not affect essentially the interpretation of the results, which were always strictly comparable within each

class, anionic, cationic or non-ionic. In each class of detergent at least one member was used which was a known substance, e.g. sodium dodecyl sulphate in the anionic class, hexadecylpyridinium bromide in the cationic class, and digitonin in the non-ionic class. The pattern of the effect of the pure member of the class on lipase was nearly always closely followed by each member of the class; thus, the behaviour of impure Teepol was closely similar to that of SDS and the behaviour of impure Lissolamine A 50 to that of hexadecylpyridinium bromide. The behaviour of the detergent towards lipase has thus been related essentially to the nature of the detergent charge.

It may be said in summarizing that concentrated solutions of nearly all the anionic and non-ionic detergents tested inhibit lipase, but although all these detergents are good emulsifying agents, stimulation of enzymic hydrolysis due to the emulsification is completely overcome by the powerful inhibitory action of the detergents on the enzyme. All cationic detergents tested, however, when used so that there is a critical detergent/triolein ratio, powerfully stimulate the hydrolysis on account of their emulsifying power. The detergent at this critical concentration can give adequate emulsification of the fatty substrate, but in this case the concentration of detergent in the aqueous phase is not great enough to inhibit the lipase. The cationic detergents inhibit lipase, either if they are used in high concentrations or if they are brought into contact with the enzyme before the triolein. Bile salts appear to be ideal emulsifying agents, since they do not inhibit triolein hydrolysis even at high concentrations. The evidence, whilst not conclusive, suggests that the essential role of the bile salts is to emulsify the fatty substrates rather than to act as true lipase activators.

SUMMARY

1. The majority of anionic detergents tested inhibited enzymic hydrolysis of triolein in high concentrations and all had no effect in dilute solutions. Liquoid Roche increased the velocity of triolein hydrolysis. Sodium dodecyl sulphate inhibited

triacetin hydrolysis but strongly stimulated tributyrin hydrolysis.

2. Cationic detergents, when used under certain conditions, markedly increased the velocity of triolein hydrolysis by lipase. The increased rate of hydrolysis produced by these detergents and that produced by bile salts were not additive. Hexadecylpyridinium bromide inhibited triacetin hydrolysis but increased the rate of tributyrin hydrolysis.

3. Non-ionic detergents, with the exception of Lubrol MO, which caused a small increase in rate, had no effect on the rate of triolein hydrolysis used in dilute solutions and inhibited lipase in concentrated solutions.

4. Cholesterol added to triolein has been found to increase the velocity of hydrolysis of triolein by lipase.

5. Experimental evidence, though not conclusive, supports the hypothesis that the main role of the bile salts is to emulsify the fatty substrates rather than to act as lipase activators.

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