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The Effect of Anionic Detergents and some Related Compounds on Enzymes

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Synthetic detergents of all types are now widely used domestically, and in many branches of medicine and industry. The biological effects of these detergents are only partially understood, and it was considered that a systematic examination of the effect of some of these detergents on enzymes would be of value. Most commercial detergents are complex mixtures and often of secret formula, but some classifications have been presented in the literature (e.g. Van Antwerpen, 1943). The present work deals only with those detergents usually referred to as the 'anionic detergents'.

In earlier investigations from this laboratory it was found that the trypanocidal drug Suramin has a strong inhibitory action on a few enzymes at pH 7.5 and a complete or almost complete inhibiting effect on many enzymes on the acid side of their isoelectric points (Wills & Wormall, 1950*a*, *b*; Wills, 1952*a*). As an extension of this work, a study has now been made of the effect of various detergents on enzymes, since preliminary experiments indicated that some detergents have an effect on enzymes somewhat similar to that of Suramin. In the investigations described here a few experiments have been made with commercial detergents, but in most cases sodium dodecyl sulphate (SDS), which is obtainable in a fairly pure state, has been used as a typical anionic detergent.

Several studies have been made of the combination of SDS with proteins (Putnam & Neurath, 1944), but few observations have been made on the effect of SDS or related compounds on enzymes. Marron & Moreland (1939) reported that surface-active substances (including a detergent) had little effect on several enzymes tested. SDS has been observed to precipitate crystalline pepsin at acid pH (Putnam & Neurath, 1944) and inactivate it at pH 4.0 (Cooper

& Bull, 1944) but only slight combination of this enzyme and detergent occurs at pH 6.5 (Lundgren, 1945). More recently, Rabinovitz & Boyer (1950) have shown that SDS inhibits the succinic oxidase system.

In the present work the effect of SDS on enzymes has been compared with the effect of several aromatic sulphonic acids and with that of some commercial detergents which are sulphonated compounds, e.g. Teepol. A preliminary report on some of these investigations has been presented (Wills, 1952*b*, 1953).

EXPERIMENTAL

Enzyme preparations and activity measurements

The methods of preparation and estimation of activity for most of the enzymes used have been previously described (Wills & Wormall, 1950*b*). Other sources and methods are described below. Where SDS or another substance was being tested for inhibitory power, a suitable concentration of this test substance was added to the enzyme mixture and the amount of water reduced accordingly.

Lipase. This was used as an aqueous extract of hog pancreas powder. This powder was prepared by extracting a pancreas with acetone and ether, and drying and pulverizing the solid in a mechanical mortar. A 5 g. sample of the powder was extracted with 50 ml. water in a mechanical shaker, and the supernatant centrifuged.

D-Amino acid oxidase. An aqueous extract of dried acetone-powder of pig kidney prepared as described by Krebs (1935) was used.

Lipase estimations. These were made by three methods.

(i) *Continuous-titration method.* Water (3.8 ml.), triacetin (0.2 ml.) and bromothymol blue (4 drops of a 0.04% solution) were mixed in a boiling tube at 18° and the pH was adjusted to 6.8 with 0.01 N-NaOH. Enzyme solution (1 ml.) was added and the pH kept at 6.8 by addition of 0.01 N-NaOH. Titration readings were taken at intervals of 1 or 2 min. for about 10 min.

(ii) *Manometric method.* The enzyme action was studied at 37° by following acid production as recorded by liberation of CO₂ from NaHCO₃. The manometers were gassed with either 95% N₂—5% CO₂ or with 100% CO₂, much more concentrated NaHCO₃ soln. being necessary in the latter case. By adjusting the concentration of NaHCO₃ the pH of the mixture was varied, the precise concentration of NaHCO₃ necessary to give the required pH being calculated by means of the Henderson-Hasselbalch equation (cf. Umbreit, Burris & Stauffer, 1949). 100% CO₂ proved more suitable for acid pH's.

For pH 7.4, NaHCO₃ solution (1 ml. of 0.06M for 5% CO₂ or 0.85 ml. of 1M for 100% CO₂), enzyme solution (1 ml.) and water (1.0 ml.) were placed in the main compartment of the Warburg flask and either neutralized triacetin (0.3 ml.) or neutralized triolein (0.4 ml.) in the side bulb.

(iii) *Willstätter's method.* This is described by Sumner & Somers (1947) and was used at 37°.

Trypsin estimations

(i) *Manometric method.* 0.06M-NaHCO₃ (1 ml.), trypsin solution (1 ml.), and water (1 ml.) were placed in the main compartment of a Warburg flask and 5% (w/v) casein (0.3 ml.) in the side bulb. The gas phase was 95% N₂—5% CO₂ and rate of CO₂ liberation was measured at 37°.

(ii) *Titration method.* Digestion was followed by the formol-titration method: 5% casein (15 ml.) or serum (15 ml.), water (20 ml.) and trypsin solution (10 ml.) were mixed in a conical flask which was placed in a water bath at 37°. Samples (10 ml.) were withdrawn at 0, 30, 60 and 90 min. and blown into 20% (v/v) formalin (10 ml.) at pH 8.2. Phenolphthalein solution (1 drop/ml.) was added and the samples were titrated to pH 8.2 with 0.05N-NaOH. The amount of NaOH required gave a measure of the progress of the digestion.

Invertase estimations. These were (i) as previously described (Wills & Wormall, 1950b), and (ii) polarimetrically. In the latter method, N acetate buffer, pH 4.0 (2.0 ml.), 20% (w/v) sucrose (20 ml.), and water (23 ml.) were mixed in a conical flask which was placed in a water bath at 37°. The reaction was started by the addition of invertase solution (5 ml.). Samples (10 ml.) were withdrawn after 0, 10, 20, 30 and 40 min. and quickly introduced into 2N-NaOH (5 ml.). The rotation of each sample was then measured in the polarimeter.

Materials. Commercial detergents were obtained from the following sources: Teepol (the sodium salt of higher alkyl sulphates) from Shell Chemicals Ltd., Liqueide Roche (sodium polyanetholsulphonate) from Roche Products Ltd., and Stergene (an alkylarylpolyoxyethanol) from Domestos Ltd.

Antrypol amine (*m*-aminobenzoyl-*m*-amino-*p*-methylbenzoyl-1-aminonaphthalene-4:6:8-trisulphonic acid) was a gift from I.C.I. Ltd.

RESULTS

Inhibition of urease by sulphonic acids

The effect of various structurally related sulphonic acids on urease was studied at pH 5.0. The compounds were tested in concentrations of 0.001M and 0.00033M and all appeared to fall fairly sharply into one of two groups. Compounds termed type A and listed in Table 1 were found to be very weak inhibitors of urease even at a concentration of 0.001M.

A consideration of the structures of these compounds shows that although these vary greatly there is one common characteristic, namely that all these compounds possess only one sulphonic acid grouping or two or more closely packed sulphonic acid groupings (as for example in the naphthalene ring). When inhibition was produced by type A compounds it was reversible with change of pH, so that adjusting the pH to 5.7 instantly abolished the inhibition.

The remaining compounds tested are termed type B and are listed in Table 2. These sulphonic acids, in contrast to type A compounds, are powerful inhibitors of urease in a concentration of 0.00033M and even in much weaker solutions. Type B compounds also vary considerably in structure, amongst themselves, but they all appear to have a common characteristic in possessing two or more sulphonic acid groups which are 'spaced' or well separated by other groups as illustrated in the formulae given in Table 2. Whether there is one or more sulphonic acid groups at each end of the molecule has no important effect on the inhibitory power, nor has the nature of the intervening part of the molecule which separates the sulphonic acid groups. Type B compounds give inhibition which is irreversible with change of pH, and give inhibition/pH curves of the type given by Suramin. Suramin, which has already been extensively investigated (Wills & Wormall, 1950b), belongs to this group of type B compounds, but it will be noticed that the half molecule of Suramin (Antrypol amine) and the terminal residue (1-naphthylamine-4:6:8-trisulphonic acid) of Suramin are both very weak inhibitors of urease and thus belong to type A.

The effect of SDS on urease

Interpretation of the results of examining the effect of SDS on urease is greatly facilitated by considering the behaviour of the SDS molecule in solution.

Micelle formation of SDS. Physico-chemical investigations of SDS solutions, by measurement of electrical conductivity, osmotic pressure and by other methods, have shown that the molecule of SDS may exist in solution either as a free ion or as a micelle. In dilute solutions, SDS exists mainly as the free ion, but in concentrated solutions the micelle predominates. This is thought to be approximately spherical and consist of several associating molecules of SDS with a negatively charged surface layer (cf. Hartley, 1936; Ralston, 1946). The transition between the two forms is fairly sharp and occurs at a critical concentration which depends on the salt concentration. For the buffer concentration used in most enzyme experiments, it is approximately 0.001M. Transition in either direction is not 100%, and there is evidence that some micelles exist even in dilute solutions.

Table 1. *Effect of aromatic sulphonic acids and SDS on urease at pH 5.0*Type A compounds (all except SDS used in a concentration of 10^{-3} M). For concentration of SDS—see text, p. 110.

Compound	Formula	Inhibition (%)
	-SO ₃ H	
Benzenesulphonic acid		0
<i>p</i> -Toluenesulphonic acid		0
Sulphanilic acid		0
Naphthionin		0
Suramin terminal group (1-naphthylamine-4:6:8-trisulphonic acid)		25
Methyl orange		0
Sodium taurocholate		0
Antropol amine		0
SDS ion	$\text{CH}_3 \cdot (\text{CH}_2)_{11} \cdot \text{SO}_4^- \text{Na}^+$	0

Table 2. *Effect of aromatic sulphonic acids and SDS on urease at pH 5.0*Type B compounds (all except SDS used in a concentration of 10^{-3} M). For concentration of SDS—see text.

Compound	Formula	Inhibition (%)
Congo red	$\text{HO}_3\text{S}-\text{[shaded box]}-\text{SO}_3\text{H}$	90
		90
Suramin		90
'Demethylated' Suramin		90
SDS micelle		90

Results of experiments which are presented later in this paper show that, in their behaviour towards urease, the SDS ion closely resembles type A compounds and the SDS micelle resembles type B compounds.

The effect of concentration. At pH 5.0 urease is completely inhibited by 0.001 M-SDS, but as the concentration of SDS is reduced inhibition falls off rapidly until it is almost zero with 0.00033 M-SDS. Comparing these results with those obtained for Suramin in equivalent concentration (Fig. 1)

it will be observed that the rate of fall of inhibition with decreasing concentration is much more rapid with SDS than it is in the case of Suramin.

The effect of pH. SDS (0.001 M) causes 100% inhibition of urease at pH 5.0, but the inhibition is markedly dependent on pH as it is in the case of Suramin (Wills & Wormall, 1950b) and falls to zero at pH 5.4. An inhibition/pH curve is obtained (Fig. 2) practically identical with that obtained using Suramin.

Fig. 1. Comparison of the effect of varying concentrations of SDS and Suramin on urease at pH 5.0.

Fig. 2. Effect of pH on inhibition of urease by 0.001 M-SDS.

Fig. 3. Reversibility of SDS inhibition of urease with change of pH from 5.0 to 5.7. Urease and SDS were left in contact for 20 min. at pH 5.0, and the estimation was carried out at pH 5.7.

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Reversibility of inhibition. Inhibition of urease by Suramin at pH 5.0 has been shown to be irreversible with change of pH, in that the enzymic activity of urease treated with Suramin at pH 5.0 cannot be restored by adjusting the pH to 5.7, a pH at which urease is not normally inhibited by Suramin (Wills & Wormald, 1950b). Other compounds of type *B* were found to behave like Suramin and produce inhibition which was not reversed by change of pH. Inhibition produced by the 'terminal residue' of Suramin (type *A* compound) was found, however, to be completely reversible when the pH was adjusted from pH 5.0 to 5.7, inhibition by 0.001 M being 37 % at pH 5.0 and zero at pH 5.7, whereas inhibition by 0.001 M Suramin is 100 % at both pH's.

In this respect SDS showed a transition between type *A* and type *B* properties. The inhibition was quite irreversible when 0.001 M-SDS was used but it was completely reversible at 0.0005 M (Fig. 3). Since in a concentration of 0.001 M, SDS will be almost completely in the micellar form and almost completely dissociated at 0.0005 M, these results indicate that inhibition produced by the micelle is irreversible (type *B*), but that produced by the free ion is reversible (type *A*).

The effect of other surface-active agents on urease

In addition to SDS the effect of some other anionic surface-active agents on urease was examined. These included Teepol, Liquoide Roche, and sodium taurocholate.

Teepol and Liquoide Roche were found to be powerful inhibitors of urease at pH 4.8; thus 0.1 % (v/v) Teepol (commercial preparation) gave 100 % inhibition, and 0.1 % (w/v) Liquoide Roche gave 95 % inhibition. In both cases inhibition fell off sharply with increasing alkalinity, and pH/inhibition curves similar to that given with SDS were obtained.

Sodium taurocholate was found to be a weak inhibitor; 0.3 % (0.061 M) produced 23 % inhibition at pH 5.0 and it gave no sharp pH/inhibition curve.

The effect of pentamidine

Pentamidine, 4,4'-diamidinodiphenoxypentane, which like Suramin is a trypanocidal drug, was observed by Guimaraes & Lourie (1951) to give a marked precipitate when mixed with Suramin, as a consequence of which the trypanocidal effect of both drugs was abolished. These experiments suggested that it might prove possible to protect enzymes against inhibition by Suramin with pentamidine.

Table 3. *Effect of 0.001 M pentamidine on urease treated with various concentrations of Suramin or SDS at pH 5.0, for varying lengths of time*

Material	Concn. (M)	Time of contact with enzyme before addition of pentamidine (min.)	Inhibition (%)
Suramin	0.00033	No pentamidine added	72
		0	0
		0.25	0
		5	30
		10	42
		20	59
SDS	0.001	No pentamidine added	99
		0	6
SDS	0.00067	No pentamidine added	76
		15	74

Marked protection against the inhibition of urease and invertase by Suramin was obtained, and in addition pentamidine was found to exert a powerful protective action against inhibition by other compounds of type B, e.g. Congo red and SDS (micelle). Although pentamidine was able to protect urease against Suramin it was not able to reverse the inhibition if it was added after Suramin had been in contact with the enzyme for an appreciable length of time.

Thus if pentamidine is added to the enzyme before, or only a few seconds after, the Suramin has been added, the full activity is maintained, but if addition of pentamidine is delayed, a slow inactivation occurs which is almost complete after 30 min. A similar result is obtained when SDS is used instead of Suramin (Table 3).

The effect of SDS on other enzymes

In addition to inhibiting urease, SDS was found to inhibit strongly several other enzymes, most of which had previously been found to be inhibited by Suramin (cf. Wills & Wormall, 1950b). The effect of SDS on different enzymes at different pH's is illustrated in Table 4. Invertase, malt α -amylase, tyrosinase and peroxidase each gave a sharply dropping pH/inhibition curve similar to that given by Suramin (Wills, 1952a). Other enzymes listed in Table 4 were inhibited over the whole pH range tested. Apart from carboxylase, which is powerfully inhibited by Suramin, enzymes which were not inhibited by 0.001 M-SDS were also those unaffected by Suramin.

In the case of urease the pH/inhibition curve for SDS was almost an exact replica of the curve for Suramin but with some other enzymes there was a tendency for the pH at which 50% inhibition occurred to be shifted slightly to the alkaline side as compared with Suramin (Table 5).

Malt β -amylase which gave a sharp pH/inhibition curve with Suramin did not give a curve with 0.001 M-SDS, inhibition being maintained at about 85% over the range pH 5.5–7.4.

The pH/inhibition curve for salivary amylase which was sharp in the case of Suramin, was smoother for SDS, shifted somewhat to the alkaline side and did not fall to zero inhibition even at alkaline pH's, e.g. 8.0 (Fig. 4).

Table 4. *Summary of the effect of 0.001 M-SDS on some enzymes*

The pH or pH range at which these enzyme tests were made was selected after a consideration of the optimum pH range of the enzyme, the stability of the enzyme at different pH's and previous experience of enzyme inhibition by Suramin.

Some enzymes inhibited by 0.001 M-SDS at the pH or pH range tested

	pH
Malt β -amylase	5.0–7.0
Salivary amylase	5.0–7.8
Pancreatic lipase	6.0–8.0
Pancreatic esterase	6.8
Papain	5.0
Rennin	5.0
Succinic oxidase	7.4
Succinic dehydrogenase	7.4
D-Amino acid oxidase	8.0

Some enzymes unaffected by 0.001 M-SDS at, or near their optimum pH's

	pH
Trypsin	7.4
Cytochrome oxidase	7.4
Carbonic anhydrase	6.8
Carboxylase	6.0
Serum cholinesterase	6.8
Papain	7.4

Some enzymes inhibited by 0.001 M-SDS below a critical pH

	Critical pH
Urease	5.2
Invertase	4.5
Malt α -amylase	6.0
Tyrosinase	4.5
Peroxidase	5.0
Xanthine oxidase	6.5

Table 5. *Comparison of the effect of pH on the inhibition of some enzymes by 0.001 M Suramin and 0.001 M-SDS*

Enzyme	pH corresponding to 50% inhibition	
	0.001 M Suramin	0.001 M-SDS
Urease	5.1	5.1
Invertase	4.1	4.4
Malt α -amylase	5.0	5.6
Malt β -amylase	5.7	*
Tyrosinase	3.9	4.6
Peroxidase	4.9	4.8

* No quickly dropping curve obtained.

Fig. 4. Comparison of the effect of 0.001M-SDS and 0.001M Suramin on salivary amylase at different pH values.

The effect of SDS and bile salts on pancreatic lipase

For a considerable time there has been controversy concerning the exact mode of action of bile salts on pancreatic lipase, the question being whether bile salts directly stimulate lipase action or whether they act solely by a detergent action emulsifying the fatty substrate (cf. Willstätter, Waldschmidt-Leitz & Memmen, 1923; Willstätter & Memmen, 1924; Glick & King, 1932; Weinstein & Wynne, 1936; Holwerda, 1938). It was considered that it would be of interest to investigate the effect of SDS and related detergents on lipase with the object of ascertaining whether such compounds could replace bile salts in stimulating the action of the enzyme. If bile salts can be so replaced, this fact would strongly substantiate the hypothesis that they are mere emulsifying agents.

Many experiments were carried out using pig pancreatic lipase with various substrates at different pH's, and using different methods of estimation. The results obtained with any one method were consistent, but did not always agree with those obtained when another method of lipase estimation was used. By the use of the manometric method with 5% CO₂ and weak bicarbonate solution, and water-soluble triacetin as substrate so that no question of emulsification is involved, it was found that 0.001M-SDS powerfully (92–75%) inhibits lipase over the pH range 6.0–8.0, no dependence of inhibition on pH being observable over this range. Sodium taurocholate (1%, w/v) also inhibited the reaction approximately 48% over the same pH range (Fig. 5) and 3% (v/v) Teepol completely inhibited triacetin hydrolysis at pH 7.4. These experiments do not substantiate the view that sodium taurocholate is an activator of lipase, at least so far as triacetin hydrolysis is concerned.

When triolein was used as substrate for the manometric method over the pH range 6.4–8.0 0.01M-SDS again strongly inhibited the hydrolysis, and so did stronger SDS solutions (e.g. 0.1M), but 1%

Fig. 5. Comparison of the effect of sodium taurocholate and SDS on pancreatic lipase using triacetin at pH 6.8 and triolein at pH 7.4.

(w/v) bile salt powerfully stimulated it. By the use of this substrate the effect of pH on the stimulating effect of bile salts on lipase activity was evident. Enzyme activity was weak in acid solutions (e.g. pH 6.4) and sodium taurocholate was able to stimulate the hydrolysis to a small extent only. The effect of the bile salt did, however, increase rapidly with rise of pH to a maximum at about pH 7.4 when a stimulation of 12 times the control rate was obtained (using 1% (w/v) sodium taurocholate) (Fig. 5).

All experiments with lipase so far described were carried out using 5% CO₂ and weak bicarbonate solutions. More acid conditions were better realized using 100% CO₂ and a stronger bicarbonate solution, and most experiments previously described were repeated under these conditions. The effects of sodium taurocholate and SDS were identical in strong and weak bicarbonate, but commercial Teepol in strong solutions (3–10%) was found markedly to stimulate triolein hydrolysis as much as 17 times the control rate at pH 7.6. This effect was sharply dependent on pH, for at pH 7.2 the same concentration of Teepol gave 100% inhibition of the hydrolysis. In weak bicarbonate solutions feeble stimulation (1.1 times the control) or no effect was observed. When Willstätter's method of estimating lipase action was used, Teepol powerfully inhibited the hydrolysis. It has not so far been possible to explain this difference, but it seems to be related in some way to the bicarbonate concentration

since with concentrations of bicarbonate between 0.02 and 0.3 M weak stimulation was observed.

Liquoide Roche or Stergene had little effect on the hydrolysis of either triacetin or triolein by lipase.

Using the continuous titration method for lipase and triacetin as substrate, SDS, in sharp contrast to the effect obtained manometrically, was found to stimulate the rate of hydrolysis to a small extent, 0.001 M-SDS producing about 1.5 times the rate of the control experiment. Sodium taurocholate (1%, w/v) had little effect on the rate. Stronger solutions of SDS, such as 0.01 M, were a little more effective than 0.001 M and stimulated the rate to about twice that of the control, but the effect was negligible with solutions containing less than 0.00067 M-SDS.

Calcium ions powerfully stimulated the rate of triacetin hydrolysis by the lipase, a rate of 3.7 times that of the control being obtained with 0.01 M- Ca^{2+} . This stimulation was abolished by 0.01 M-SDS, an effect which may be due to precipitation of calcium dodecyl sulphate.

The effect of SDS on the succinic oxidase system

Various compounds have, from time to time, been discovered which (a) totally inhibit the complete succinic oxidase system, (b) have no effect on cytochrome oxidase, and (c) only partially inhibit succinic dehydrogenase. Bile salts, naphthoquinones and hydroxystilbene are some examples of such compounds which may be quoted (cf. Slater, 1949). The inhibition produced by such compounds is cited as evidence that there exist intermediate factors or enzymes other than succinic dehydrogenase, cytochrome c, and cytochrome oxidase which link succinate to oxygen.

SDS was found to belong to this class of compound (Rabinovitz & Boyer, 1950) and the present work supports these findings (Fig. 6). It was thought possible, however, that the weak inhibition (relative to that of the whole succinic oxidase system) obtained when investigating succinic dehydrogenase might be due to combination of the basic methylene blue (MB), with the acidic SDS, although Rabinovitz & Boyer (1950) rejected this view. This possibility was tested by examining the effect of 0.001 M-SDS alone and with the addition of MB in the same concentration as is used in succinic dehydrogenase experiments, on yeast fermentation. SDS (0.001 M) alone totally inhibited fermentation but addition of 0.08% (w/v) MB reduced this inhibition almost to zero (Fig. 7). MB was also found to reduce SDS inhibition of urease and D-amino acid oxidase. These experiments lead to the conclusion that it is very likely that SDS actually does act on succinic dehydrogenase and that the relatively weak inhibition observed for succinic dehydrogenase is merely an artifact caused by combination of MB with SDS.

The effect of SDS on invertase

Invertase is completely (100%) inhibited by 0.001 M-SDS at pH 4.0. In contrast to urease, inhibition does not fall rapidly with decreasing concentration of inhibitor, 0.0002 M-SDS giving nearly 100% inhibition and 0.0001 M, 39%.

Fig. 6. Comparison of the effect of SDS on the succinic oxidase system, succinic dehydrogenase and cytochrome oxidase (0.001 M-SDS and 0.08% methylene blue).

Fig. 7. Effect of 0.001 M-SDS and 0.08% methylene blue on the fermentation of glucose by yeast.

Although a sharp pH/inhibition curve was obtained, inhibition by 0.001M-SDS falling to zero at pH 4.7, the activity of the enzyme could not be regained by changing the pH from 4.0 (100% inhibition) to pH 5.0 and was thus irreversible. In this respect invertase resembles urease, but the effect of SDS on invertase is in sharp contrast to the effect of Suramin which gives an inhibition which is reversible with pH.

The addition of the products of the hydrolysis, glucose and fructose, to invertase inhibited by 0.0002M-SDS gave interesting results. Glucose produced no effect in any concentration but 0.067M fructose practically overcame the inhibition and restored the activity (Fig. 8). Under the conditions of the experiment neither sugar had much effect on the rate of the control. Using 0.0002M-SDS the effect of fructose fell off with a decrease in its concentration, a 0.01M solution only producing a 12% reduction of inhibition and the effect of 0.002M was not detectable. The addition of a mixture of 0.04M glucose and 0.04M fructose to the inhibited enzyme, produced the same result as 0.04M fructose alone. Galactose resembled glucose in having no effect on the inhibition.

The effect of SDS on milk-clotting enzymes

SDS, like Suramin, inhibited milk clotting by papain and rennin. SDS (0.005M) completely inhibited in the case of both enzymes, whilst 0.001M caused 50% inhibition of the papain action and

Fig. 8. Comparison of the effect of 0.067M glucose and 0.067M fructose on invertase inhibited by 0.0002M-SDS at pH 4.0. The lowest curve represents results which were obtained with and without glucose.

65% inhibition of rennin action. As in the case of Suramin it is believed that SDS combines with the substrate (casein) rather than with the enzyme.

The effect of SDS on trypsin

The effect of SDS on trypsin was investigated using a variety of substrates. Dilute solutions (0.001M) of SDS had little or no effect with any substrate, but more interesting results were obtained when 0.01M-SDS was used.

Casein. SDS (0.01M) caused 10% inhibition of the hydrolysis of a 5% casein solution by trypsin.

Horse serum. The hydrolysis of the natural serum proteins by trypsin is very slow, but the reaction is strongly accelerated when the proteins are denatured (Haurowitz, Tunca, Schwerin & Goksu, 1945). Denaturation in the present series of experiments was normally accomplished by keeping the serum at 100° for 15 min. and then dispersing the coagulum with an equal volume of water in a Waring Blendor. SDS (0.01M) was found to markedly speed up the rate of digestion of native serum but it could also accelerate, though to a smaller extent, the hydrolysis of denatured serum proteins (Fig. 9).

The effect of trypsin and 0.01M-SDS on the insoluble denatured serum was to effect a rapid clarification of the solution which was complete after about 20 min. Neither trypsin nor SDS alone

Fig. 9. Effect of 0.01M-SDS on trypsin using native and denatured horse-serum proteins as substrates. Estimation by formol titration.

could effect this clarification even after 4 hr. The concentration of SDS used, 0.01M, seemed to be about optimum under the conditions of these experiments, since stronger solutions (e.g. 0.02M) did not give an enhanced effect and stimulation fell off rapidly with decreasing concentration and was negligible at 0.002M. When the serum was denatured by treatment with acid the results obtained were practically identical with those obtained using heat-denatured serum.

SDS at 0.01M was also found to accelerate the rate of tryptic digestion of horse-serum albumin, ovalbumin and to a small extent that of fibrin, but it had very little or no effect on the rate of digestion of these proteins when denatured.

DISCUSSION

The results of investigating the effect of a series of aromatic sulphonic acids on urease have, in general, supported the conception of a bridging mechanism of inhibition which was suggested as a possible explanation for the action of Suramin on urease (Wills & Wormall, 1950b). A consideration of Tables 1 and 2 will show that molecular size of the sulphonic acid cannot account for the inhibition produced. For example, the molecular weights of Congo red and Antrypol amine are very nearly the same, 696 and 701, respectively, and yet at pH 5.0 Congo red is at least 100 times more powerful an inhibitor than is the Antrypol amine. It is clear that the question of molecular size bears no relation to inhibition and some other explanation must be offered.

Type *B* compounds would probably combine with two adjacent or fairly close basic groupings of the enzyme in a manner similar to that suggested for Suramin by Wills & Wormall (1950b). These sulphonic acids, so combined with the enzyme, are likely to produce powerful steric blocking of the active centres and thus effect powerful inhibition. Inhibition of urease by type *B* compounds cannot be reversed by adjusting the pH or by adding pentamidine. This irreversibility suggests that binding of the enzyme's basic groups by this bridge type of combination causes distortion of the molecule and thus irreversible denaturation. The fact that SDS can act as a powerful protein-denaturing agent has been previously established (Putnam, 1948).

Type *A* sulphonic acids, on the other hand, would probably combine only at a single enzyme basic grouping. Results of experiments using type *A* compounds suggest that this type of combination offers little or no opposition to enzyme action and that there is little steric resistance to substrate molecules reaching the active centres. Furthermore, this combination does not strain the enzyme mole-

cule since type *A* sulphonic acids appear to be easily liberated from the enzyme complex when the pH is made alkaline with respect to the isoelectric point of urease.

In dilute solutions, weaker than 0.001M, the greater portion of the SDS molecules exist in solution as free ions which are weak inhibitors and may be classed with type *A* compounds. At a concentration of 0.001M, and in stronger solutions, SDS takes on the micellar form, becomes a powerful inhibitor of urease and may be classed as type *B* if the $-\text{SO}_3$ groupings at the surface of the micelle are considered analogous to the spaced sulphonic acid groupings of type *B* sulphonic acids. Inhibition falls more rapidly with decreasing concentration in the case of SDS than with Suramin owing to the dissociation of the micelle into free ions. Dissociation cannot occur in the case of Suramin, but artificial dissociation of Suramin, i.e. the use of the half molecule, causes a sharp decline of inhibition (Tables 1 and 2). Experiments on reversibility of SDS inhibition support these views, since inhibition of urease by SDS solutions less concentrated than 0.001M is reversible, whilst that of solutions more concentrated than 0.001M is irreversible.

The biological action of a compound does not necessarily bear any relation to its action as an inhibitor of enzymes. For example, a list of a few compounds (Table 2) which behave in practically the same way towards urease, includes a dyestuff (Congo red), a trypanocidal drug (Suramin) and a detergent (SDS).

Pentamidine almost certainly protects urease against type *B* compounds by combining with the sulphonic acid groups to give a fairly stable, ring-like compound, bound by salt linkages. It might be regarded as a 'basic analogue' of type *B* compounds, and is the most powerful protective agent for enzymes against inhibition by type *B* compounds, some proteins excepted, that has so far been discovered. This fact suggests that its basic groupings may be a model of those found in the protein, and that the spacing of these basic groupings may closely resemble that in the enzyme proteins. One amidine grouping in a compound is not sufficient to protect enzymes against inhibition by type *B* compounds, since arginine will not protect urease against Suramin (Wills & Wormall, 1950b), and no protection is obtained using basic compounds such as adenine, guanine, histidine and lysine (Wills, unpublished work, 1950).

As in the case of experiments with Suramin, it has been found that urease is a useful model enzyme, and that many other enzymes resemble it in their reaction to SDS. In the many cases where the effect of SDS closely resembles that of Suramin it may be inferred that inhibition by these two substances occurs by a common mechanism, namely

the binding of acidic groups of the inhibitor with basic enzyme groups. In some cases, however, SDS is a more powerful inhibitor than is Suramin, especially with regard to its effect on certain enzymes on the alkaline side of their isoelectric points. The pH/inhibition curve for SDS does not always fall so sharply as in the case of Suramin and with some enzymes, e.g. α -amylase and salivary amylase, does not fall to zero inhibition at any pH, a fact which suggests that for these enzymes SDS inhibition may be more complex than is that of Suramin. A probable explanation of this difference is that in the case of SDS, in addition to simple ionic binding, there is the possibility of association of the long hydrocarbon chains with the peptide chains of the proteins. This is known to occur (cf. Putnam, 1948).

In the case of invertase, competition between the molecule of SDS and fructose suggests that each is attracted to a basic grouping, probably an amino grouping in the active centre of the enzyme. The view that fructose and acidic inhibitors were attracted to basic groupings in the active centre of the invertase was proposed by Quastel & Yates (1936) as a result of investigations on the effect of acid dyes on invertase. Their results, however, did not give such clear-cut evidence for the theory as do the SDS experiments described here. For example, they found that 0.22M fructose reduced inhibition produced by 1.19×10^{-4} (w/v) Soluble blue from 66.5 to 52%, and of 1.14×10^{-4} (w/v) Trypan red from 78.6 to 62.7%, whereas this concentration of fructose reduced inhibition produced by 0.0002M-SDS from 100% to practically zero. Some experiments with Suramin gave results which were similar to those which Quastel & Yates (1936) obtained with acid dyes; for example 0.067M fructose could only reduce the inhibitory effect of 0.001M Suramin from 67 to 62%. SDS thus appears to have a very specific affinity for certain basic groupings in the active centre of invertase which bind the fructose fragment of sucrose.

Attempts to replace bile salts as activators of lipase action with detergents were not generally successful. Although the detergent may be effective as an emulsifying agent, this action may be nullified by a direct inhibitory action on the enzyme. Nearly all the detergents tested did, however, inhibit the hydrolysis of triacetin by lipase. The stimulatory effect of strong Teepol solutions on the hydrolysis of triolein by lipase at pH 7.6 is not easy to explain and may be an artifact of the method of estimation. The dependence of the stimulation on the concentration of bicarbonate may be significant, however, especially in view of the fact that the bicarbonate concentration of the human duodenum may be as much as 0.1M (Harrison, 1947). In the present series of experiments Teepol gave no stimulation of

the rate of hydrolysis in 0.06M-NaHCO₃ but powerfully stimulated in 0.28M-NaHCO₃.

The effect of SDS on the hydrolysis of serum proteins by trypsin may be readily explained, since it is known that SDS is a powerful denaturing agent (Putnam, 1948) and also that it stimulates acid hydrolysis of proteins. Trypsin, in general, hydrolyses denatured proteins much more rapidly than native proteins (Haurowitz *et al.* 1945) and SDS greatly accelerates protein hydrolysis by its denaturing action. It is probable that, in addition, SDS has some direct effect on the peptide linkage of proteins since it accelerates acid hydrolysis (Steinhardt, 1941), and actual combination of SDS with peptide linkages may occur after saturation of the basic groups of the protein.

To summarize, the results of the investigations on the effect of SDS on enzymes are in good agreement with those obtained from the study of the combination of SDS with inert proteins, many results of which are summarized by Putnam (1948). Enzymes for the most part do not appear to react with these anionic detergents in a very different manner from other proteins, but a study of enzyme inhibition can often give a more sensitive and precise means of studying detergent-protein combination than can conventional methods using inert proteins.

SUMMARY

1. The effect of several aromatic sulphonic acids on urease has been studied at pH 5.0, and, depending as to whether they are powerful or weak inhibitors they have been divided into two groups. Inhibition has been related to the structure of the sulphonic acids.

2. Sodium dodecyl sulphate (SDS) (0.001M) completely inhibits urease at pH 5.0, but inhibition falls off sharply with increased alkalinity and decreasing concentration of SDS. Inhibition is irreversible with 0.001M but reversible with 0.0005M-SDS.

3. Commercial detergents Teepol and Liquioide Roche act on urease in a similar manner to SDS, but sodium taurocholate is only a weak inhibitor.

4. Pentamidine protects urease against inhibition by Suramin, Congo red and SDS at pH 5.0 but it does not reverse inhibition if these compounds have prior contact with the enzyme.

5. Most enzymes tested are inhibited by SDS if the pH is suitably adjusted. Many give sharp pH/inhibition curves similar to those given by Suramin, but the pH of 50% inhibition is sometimes more alkaline than that obtained with Suramin.

6. Sodium taurocholate, SDS and Teepol inhibit the hydrolysis of triacetin by pancreatic lipase. Sodium taurocholate stimulates, but SDS inhibits triolein hydrolysis. Under certain conditions Teepol stimulates triolein hydrolysis.

7. SDS (0.001M) inhibits succinic dehydrogenase to a less extent than it does the whole succinic oxidase system, possibly owing to its combination with MB. SDS has no effect on cytochrome oxidase.

8. SDS (0.0002M) strongly inhibits invertase at pH 4.0. This inhibition can be completely abolished by 0.067M fructose but not by glucose.

9. SDS has little effect on the hydrolysis of casein by trypsin, but it strongly stimulates digestion of native serum proteins and to a less degree that of the denatured proteins.

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Dietary Fat and Body Fat: the Composition of the Back Fats of Pigs Fed on a Diet Rich in Cod-liver Oil and Lard

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In a recent study (Garton, Hilditch & Meara, 1952) of the depot fats of a pig fed on a diet rich in whale oil it was found that apparently unchanged whale-oil glycerides were present in the depots. However, since the whale oil was ingested in very large amounts and represented practically all the fat in the diet, the depot glycerides which resembled whale oil would have been substantially the same whether they had been hydrolysed and resynthesized during passage from the intestine to the lymphatics or whether they had been absorbed intact. That the pig is capable of depositing dietary fatty acids in its fat depots is well known (for a review see Hilditch, 1947, pp. 99–101), though it is not known to what extent dietary triglycerides are absorbed as such and deposited in the fat depots.

The present study was undertaken to obtain evidence in this direction by feeding a diet containing a mixture of lard and cod-liver oil to two young pigs and then studying the composition of the resultant depot fats. If intestinal hydrolysis occurred to any appreciable extent, followed by resynthesis in the intestinal wall or elsewhere, the component fatty acids characteristic of cod-liver oil (especially the polyethenoid C₂₀–C₂₂ acids) and of lard would mix and result in the formation of depot glycerides which would not be separable by low-temperature crystallization into groups of glycerides resembling lard and cod-liver oil, respectively. As described below, such a separation was, however, effected, although the cod-liver oil had undergone oxidative changes, and it seems therefore that