a phospholipase *D*. Consequently, the 'glyceryl-phosphorylester intermediate' route probably represents the major if not the sole pathway of phosphoglyceride catabolism in the liver.

Although lysolecithin would be an intermediate in the route

its presence has never been demonstrated with certainty in fresh mammalian tissues. However, an accumulation of this intermediate may be undesirable for the animal because of its well-known property of disrupting cell membranes. Consequently, an active phospholipase *B* would be a necessity, especially in tissues which can rapidly catabolize phosphoglycerides.

SUMMARY

1. The presence of an active phospholipase *B* has been demonstrated in glycerinated extracts of acetone-dried rat and sheep liver, with lysolecithin as a substrate.

2. The liver extracts can also break down lysophosphatidylethanolamine when this is added in a soluble form.

3. Maximal hydrolysis of lysolecithin was obtained at pH 6.2 and a substrate concentration between 2 and 3.5×10^{-3} M.

4. The enzyme is resistant to most enzyme inhibitors. However, Hg^{2+} , Cu^{2+} and Zn^{2+} ions are inhibitory, as is saturation of the incubation medium with ether.

5. Lysolecithin breakdown has also been observed to occur with extracts from acetone-dried rat kidney, brain, heart, spleen and whole blood, but not with skeletal muscle. Phospholipase *B* is also present in sheep kidney and thyroid.

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Protection of Succinic Dehydrogenase Thiol Groups by Fluoride and Phosphate

BY A. O. M. STOPPANI AND J. A. BRIGNONE

Department of Biochemistry, School of Medicine, University of Buenos Aires

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The biological oxidation of succinate is inhibited by fluoride (Batelli & Stern, 1911). Several explanations have been proposed for fluoride action, e.g. competition of fluoride with succinate for succinic dehydrogenase [D. J. O'Kane, unpublished data quoted by Potter & Schneider (1942)]. Phosphate is necessary for the inhibitory action of fluoride (Slater & Bonner, 1952), and kinetic deductions led these authors to postulate the formation of a

slightly dissociated inactive complex made up of fluoride, dehydrogenase and phosphate in which fluoride and phosphate compete with succinate for the enzyme active centre.

Succinic dehydrogenase is protected from thiol reagents by succinate and competitors of succinate, e.g. malonate (Hopkins, Morgan & Lutwak-Mann, 1938; Potter & DuBois, 1943; Barron & Singer, 1945), oxaloacetate, suramin and 1-naphthylamine

4:6:8-trisulphonate (Stoppani & Brignone, in preparation). A similar protection of the enzyme by the joint action of fluoride and phosphate would support the theory of the fluoride-enzyme-phosphate complex.

EXPERIMENTAL METHODS

Succinoxidase. This enzyme was obtained from pig-heart muscle as described by Bonner (1954). The acid precipitation took place at 4° and the precipitate was spun off at 2° and 2500 rev./min. in an International Refrigerated PR2 centrifuge. The enzyme was suspended in five times its volume of borate buffer (Palitzsch's buffer, as described by Clark, 1928), 0.1M, pH 7.3, and re-centrifuged for 30 min. at 13 000 rev./min. in a Servall SSI centrifuge. The succinoxidase was then suspended in borate buffer (0.2M) at pH 7.3. In this manner, the phosphate remaining from the heart-muscle initial extraction was greatly reduced. The residual free phosphate present in each succinoxidase preparation was estimated according to Fiske & Subbarow (1925) in the supernatant after precipitation of succinoxidase with 3.3% (w/v) trichloroacetic acid (first step in measuring the dry weight of succinoxidase, according to Slater, 1949a).

Enzyme activities. Succinic dehydrogenase and succinoxidase were measured manometrically at 37°, as described by Slater (1949a).

General procedure. Unless stated otherwise, the succinoxidase suspensions (0.8–0.9 ml.) were made up to 1.0 ml. with 0.1–0.2 ml. of ion mixture and 0.01–0.05 ml. of thiol reagent solution (final pH, 7.3). After standing at 37° the required time, the enzyme suspensions were diluted with phosphate buffer or distilled water and 0.3 ml. portions placed in the side bulb of Warburg vessels which in their main compartment contained the reagents necessary to measure succinic dehydrogenase. After 10 min. equilibration, enzyme activities were recorded. Controls not treated with sulphhydryl reagents or ion mixtures were measured simultaneously. Succinoxidase inactivation with BAL was carried out as described by Slater (1949b).

Reagents. The following were used: iodoacetic acid and ethylenediaminetetraacetate (disodium salt, pure) from British Drug Houses Ltd.; o-iodosobenzoic acid, prepared according to Askenasy & Meyer (1893); 3-amino-4-hydroxyphenylarsenoxide hydrochloride (mapharside), from Parke Davis and Co.; 3-melaminylphenylarsenoxide (melarsen oxide), from May and Baker Ltd.; N-ethylmaleimide, from Schwarz Laboratories, Inc.; 2,3-dimercaptopropanol (BAL), from Boots Pure Drug Co. Other reagents were of analytical grade.

To avoid the possible presence of pyrophosphate in phosphate buffers these were prepared by adjusting to pH 7.32 with NaOH–10% (w/v) phosphoric acid boiled under reflux for 10 hr. The final concentration of phosphate in these buffers was measured according to Fiske & Subbarow (1925).

Expression of results. The amount of succinoxidase used in the experiments is represented by the weight (mg.) of the protein defatted and dried according to Slater (1949a).

The effect of fluoride and other ions on the inactivation of succinic dehydrogenase by sulphhydryl reagents has been

calculated from equation (1), where P is the protection (%) of succinic dehydrogenase, I_{tr} the inhibition (%) of succinic dehydrogenase by the thiol reagent, and $I_{i, tr}$ the relative diminution (%) of succinic-dehydrogenase activity in the presence of thiol reagent, fluoride and/or other ions. All inhibitions have been calculated in relation to the respective controls:

$$P = \{I_{tr} - I_{i, tr}\} / I_{tr} \quad (1)$$

RESULTS

Effect of fluoride and phosphate on the action of thiol reagents. As shown in Table 1, succinic dehydrogenase is inactivated by arsenicals, o-iodosobenzoate, iodoacetate and N-ethylmaleimide. This is consistent with previous observations by Hopkins & Morgan (1938), Potter & DuBois (1943), Barron & Singer (1945) and Slater (1949c). The effect of N-ethylmaleimide, which reacts quantitatively with thiols (Tsao & Bailey, 1953), could be inferred from that of maleate, also an inhibitor of succinic dehydrogenase (Morgan & Friedmann, 1938).

The inactivation of succinic dehydrogenase by mapharside and by the lower concentrations of o-iodosobenzoate and N-ethylmaleimide, is decreased in the presence of phosphate. Fluoride diminishes slightly the action of mapharside, melarsen oxide and the lower concentration of o-iodosobenzoate. It is difficult to decide whether this action of fluoride is dependent on the small concentration of residual phosphate of the succinoxidase preparations.

Fluoride and phosphate produce together a remarkable diminution in the action of all the sulphhydryl-binding compounds tested. When there is protection of succinic dehydrogenase by either fluoride or phosphate, the protection obtained in association always greatly exceeds the numerical addition of their individual actions. At a fixed concentration of phosphate, the protection given to succinic dehydrogenase increases when the amount of fluoride is increased, whereas with fluoride concentration constant the protection increases with the amount of phosphate (Table 2). This recalls the experiments on succinic-dehydrogenase inhibition by fluoride and phosphate reported by Slater & Bonner (1952).

Specificity of fluoride action. The ability of fluoride to protect succinic dehydrogenase in the presence of phosphate seems to be unique. Table 3 shows that equal concentrations of chloride and bromide rather increase the effect of thiol reagents on the enzyme; iodide has a slight protecting action and thiocyanate scarcely affects the extent of the inactivation. With the lower concentration of phosphate (due to the residual phosphate in succinoxidase) the halogen ions exert a very weak protection on the enzyme with the exception, however, of chloride.

Table 1. *Protection of succinic dehydrogenase by fluoride in the presence of phosphate*

Succinoxidase (1.0 ml.) treated with inhibitors and additions as shown was incubated at 37° for 40 min. (for melsarsen oxide) or 30 min. (for other reagents) and was then diluted with 4.0 ml. of 0.4M phosphate, pH 7.3, or 5.0 ml. of distilled water (for melsarsen oxide). Succinic-dehydrogenase activity was measured in 0.3 ml. of this extract.

Succinoxidase (mg./ml.)	Residual phosphate of succinoxidase (mm)	Additions	Succinic dehydrogenase	
			Inhibition (%)	Protection (%)
0.17 mm mapharside				
13.4	—	None	79.0	—
13.4	—	0.064M phosphate	62.5	20.8
13.4	—	4.0 mm fluoride	63.1	20.1
13.4	—	0.064M phosphate + 4.0 mm fluoride	10.3	86.6
0.23 mm melarsen oxide				
26.0	0.5	None	80.0	—
26.0	0.5	0.064M phosphate	75.2	6.0
26.0	0.5	4.0 mm fluoride	62.0	22.5
26.0	0.5	0.064M phosphate + 4.0 mm fluoride	0.0	100.0
8.2 mm iodoacetate				
6.9	0.5	None	76.1	—
6.9	0.5	0.080M phosphate	70.5	7.3
6.9	0.5	4.0 mm fluoride	70.5	7.3
6.9	0.5	0.080M phosphate + 4.0 mm fluoride	- 6.9	108.7
0.21 mm <i>o</i> -iodosobenzoate				
15.1	0.3	None	75.0	—
15.1	0.3	0.064M phosphate	43.5	42.0
15.1	0.3	4.0 mm fluoride	53.8	28.2
15.1	0.3	0.064M phosphate + 4.0 mm fluoride	11.1	85.0
0.42 mm <i>o</i> -iodosobenzoate				
15.1	0.3	None	93.0	—
15.1	0.3	0.064M phosphate	92.0	1.1
15.1	0.3	4.0 mm fluoride	94.5	- 1.6
15.1	0.3	0.064M phosphate + 4.0 mm fluoride	8.5	90.7
0.36 mm <i>N</i> -ethylmaleimide				
12.0	0.1	None	100.0	—
12.0	0.1	0.060M phosphate	86.5	13.5
12.0	0.1	4.0 mm fluoride	96.0	4.0
12.0	0.1	0.060M phosphate + 4.0 mm fluoride	25.0	75.0

Table 2. *Effect of several concentrations of fluoride and phosphate on succinic-dehydrogenase inactivation by N-ethylmaleimide*

1.0 ml. of enzyme suspension (15.2 mg. of succinoxidase) was treated at 37° for 30 min. with 0.48 mm N-ethylmaleimide, 0.08M phosphate, pH 7.3 (Expt. A), or 10.0 mm fluoride (Expt. B) and additions as shown, and then diluted with 4.0 ml. of distilled water. Succinic dehydrogenase was measured in 0.3 ml. of this extract.

Expt.	Additions	Succinic-dehydrogenase	
		Inhibition (%)	Protection (%)
	Fluoride concn. (mm)		
A	None	83.5	—
	10.0	7.0	91.5
	5.0	29.0	65.0
	1.0	68.5	18.0
	0.5	72.0	13.8
	Phosphate concn. (mm)		
B	80.0	18.2	81.8
	20.0	32.4	67.6
	4.0	56.0	44.0
	0.2	100.0	—

Requirement of phosphate for fluoride action. The concentration of phosphate needed to allow the protection of succinic dehydrogenase by fluoride appears to mean a significant increase of ionic strength and molar concentration in the medium where the enzyme reacts with the thiol detectors. This raises the question whether phosphate potentiates fluoride in a non-specific manner, i.e. through changes in the activity coefficient of fluoride ions and the ion groups of succinic dehydrogenase able to bind fluoride. However, the following facts conflict with that hypothesis: (1) sulphate and arsenate potentiate fluoride, but far less than the same concentration of phosphate (Tables 1-4); (2) the change in molarity (and ionic strength) brought about by the addition of phosphate is less striking than appears at first. In the experiments shown in Tables 1 and 2, 0.8 ml. of succinoxidase in 0.2M borate buffer was treated with 0.2 ml. of ion mixture and 0.02 ml. of inhibitor solution, which means that the maximal increase in molarity was from 0.20 to 0.28; (3) potassium nitrate or sodium chloride, in amount giving the same ionic strength

as phosphate, scarcely affects the action of fluoride (Table 5). There is a certain protection of succinic dehydrogenase by nitrate, which, however, does not potentiate fluoride, as the effect of nitrate and fluoride together exceeds only slightly the sum of their individual actions.

The ionic strength of the ion mixtures added to the succinoxidase-borate suspension has been calculated as half the sum of the products of each ion molarity by the square of its valency (Lewis & Randall, 1923); for the present purpose this may be considered accurate enough. The action of fluoride in media of equal ionic strength is obtained by comparing the experiments with 0.163 M nitrate and fluoride (Table 5), and the experiments with 0.064 M phosphate, arsenicals and *o*-iodosobenzoate, where the ionic strength added to the succinoxidase-borate solution was about 0.16. The action of fluoride in media of equal molarity may be seen by comparing the experiments with 0.060 M chloride (Table 5) with the experiments with 0.060 M phosphate and *N*-ethylmaleimide as inhibitor.

The possibility of replacing phosphate by sulphate, though limited, suggests that phosphate and

Table 3. *Effect of halogen ions on succinic dehydrogenase inactivation by o-iodosobenzoate*

Enzyme suspension (1.0 ml.) (15.3 mg. of succinoxidase) was treated with *o*-iodosobenzoate, phosphate and additions as shown, for 20 min. at 37°, and was then diluted with 4.0 ml. of distilled water. Succinic-dehydrogenase activity was measured in 0.3 ml. of this extract.

Additions	Succinic-dehydrogenase	
	Inhibition (%)	Protection (%)
0.28 mM <i>o</i> -iodosobenzoate + 0.08 M phosphate		
None	62.0	—
4.0 mM fluoride	9.0	85.5
4.0 mM chloride	79.0	-27.4
4.0 mM bromide	78.6	-26.8
4.0 mM iodide	56.5	8.8
4.0 mM thiocyanate	60.0	3.2
0.28 mM <i>o</i> -iodosobenzoate + 0.2 mM phosphate		
None	100.0	—
4.0 mM fluoride	88.2	11.8
4.0 mM chloride	95.5	4.5
4.0 mM bromide	89.0	11.0
4.0 mM iodide	84.5	15.5
4.0 mM thiocyanate	88.0	12.0

Table 4. *Protection of succinic dehydrogenase by fluoride in the presence of sulphate and arsenate*

Succinoxidase (1 ml.) was treated at 37° with inhibitors and additions as shown for 30 min. (Expt. A) or 20 min. (Expt. B) and then diluted with 4.0 ml. of 0.4 M phosphate, pH 7.3 (Expt. A) or 4.0 ml. of distilled water (Expt. B). Succinic dehydrogenase was measured in 0.3 ml. of this extract.

Expt.	Succinoxidase (mg.)	Residual phosphate in succinoxidase (mm)	Additions	Succinic-dehydrogenase	
				Inhibition	Protection
				(%)	(%)
			0.17 mm mapharside		
A	13.4	—	None	79.0	—
	13.4	—	0.040 M sulphate	78.5	0.6
	13.4	—	4.0 mm fluoride	63.1	20.1
	13.4	—	0.040 M sulphate + 4.0 mm fluoride	36.0	54.5
			0.42 mm <i>o</i> -iodosobenzoate		
	15.1	0.3	None	93.0	—
	15.1	0.3	0.064 M sulphate	93.5	0.5
	15.1	0.3	4.0 mm fluoride	94.5	-1.6
	15.1	0.3	0.064 M sulphate + 4.0 mm fluoride	49.0	47.2
			0.36 mm <i>N</i> -ethylmaleimide		
	12.0	0.1	None	100.0	—
	12.0	0.1	0.060 M sulphate	90.6	9.4
	12.0	0.1	4.0 mm fluoride	96.0	4.0
	12.0	0.1	0.060 M sulphate + 4.0 mm fluoride	40.0	60.0
			0.36 mm <i>N</i> -ethylmaleimide		
B	12.0	1.2	None	97.0	—
	12.0	1.2	0.080 M arsenate	98.0	-1.0
	12.0	1.2	4.0 mm fluoride	89.0	8.2
	12.0	1.2	0.080 M arsenate + 4.0 mm fluoride	48.5	50.0
			12.0 mm iodoacetate		
	12.0	1.2	None	90.5	—
	12.0	1.2	0.080 M arsenate	94.0	-3.9
	12.0	1.2	4.0 mm fluoride	89.0	1.7
	12.0	1.2	0.080 M arsenate + 4.0 mm fluoride	46.5	48.5

arsenate potentiate fluoride through their bivalent anions.

Influence of sulphate, nitrate and chloride on inhibition of succinic dehydrogenase by fluoride. It is known that phosphate and arsenate are required if fluoride is to inhibit succinic dehydrogenase (Slater & Bonner, 1952). The action of phosphate has been now compared with that of sulphate, nitrate and chloride (Table 6). As was expected, sulphate

increases fluoride action but to a less extent than does phosphate. Nitrate and chloride have no action in amounts giving the same ionic strength as phosphate, e.g. the experiment with 0.10M phosphate and 0.26M chloride (added ionic strength 0.26) and the experiment with 0.066M phosphate and 0.158M nitrate (added ionic strength about 0.16). Incidentally, the strong inhibition of succinic dehydrogenase by the univalent anions may be noted.

Table 5. *Action of fluoride on succinic dehydrogenase in the presence of nitrate and chloride*

Succinoxidase (1 ml.) was treated with inhibitors and additions as shown for 30 min. at 37° and then diluted with 5.0 ml. (for iodoacetate) or 4.0 ml. (for other reagents) of distilled water. Succinic dehydrogenase was measured in 0.3 ml. of this extract.

Succinoxidase (mg.)	Residual phosphate in succinoxidase (mm)	Additions	Succinic-dehydrogenase	
			Inhibition (%)	Protection (%)
		0.28 mm <i>o</i> -iodosobenzoate		
15.3	0.21	None	95.0	—
15.3	0.21	0.163M nitrate	81.5	14.2
15.3	0.21	4.0 mm fluoride	86.6	8.8
15.3	0.21	0.163M nitrate + 4.0 mm fluoride	67.5	29.0
		8.0 mm iodoacetate		
18.4	0.27	None	100.0	—
18.4	0.27	0.120M chloride	96.5	3.5
18.4	0.27	4.0 mm fluoride	96.0	4.0
18.4	0.27	0.120M chloride + 4.0 mm fluoride	96.5	3.5
		0.28 mm <i>o</i> -iodosobenzoate		
11.3	0.14	None	94.0	—
11.3	0.14	0.060M chloride	97.0	-3.2
11.3	0.14	4.0 mm fluoride	95.0	-1.1
11.3	0.14	0.060M chloride + 4.0 mm fluoride	94.0	0.0
		0.24 mm <i>N</i> -ethylmaleimide		
11.3	0.14	None	100.0	—
11.3	0.14	0.060M chloride	95.5	4.5
11.3	0.14	4.0 mm fluoride	100.0	0.0
11.3	0.14	0.060M chloride + 4.0 mm fluoride	86.5	13.5
		0.28 mm <i>o</i> -iodosobenzoate		
15.3	0.21	None	97.5	—
15.3	0.21	0.163M chloride	87.5	10.3
15.3	0.21	4.0 mm fluoride	88.2	9.5
15.3	0.21	0.163M chloride + 4.0 mm fluoride	85.5	12.3

Table 6. *Effect of phosphate, sulphate, nitrate and chloride on succinic-dehydrogenase inhibition by fluoride*

0.021M succinate; 0.005M methylene blue; 0.006M cyanide; 0.07M borate buffer, pH 7.3. Air as the gas phase. 37°.

Succinoxidase (mg.)	Additions	Succinic- dehydrogenase inhibition
		(%)
1.7	10.0 mm fluoride	7.6
	0.1M phosphate	13.8
	10.0 mm fluoride + 0.1M phosphate	43.5
	0.1M sulphate	20.0
	10.0 mm fluoride + 0.1M sulphate	45.5
	0.26M chloride	56.2
	10.0 mm fluoride + 0.26M chloride	58.6
	10.0 mm fluoride	13.7
1.9	0.066M phosphate	0.0
	10.0 mm fluoride + 0.066M phosphate	41.0
	0.158M nitrate	28.5
	10.0 mm fluoride + 0.158M nitrate	31.0

This has already been observed by Batelli & Stern (1911) with chloride, and by Slater & Bonner (1952) with nitrate.

Inactivation of succinoxidase with BAL in the presence of fluoride. The oxidation of succinoxidase with BAL inactivates a factor linking the dehydrogenase to the cytochrome system (Slater, 1949b). It is still under discussion whether the BAL-sensitive factor (Slater's factor) is a distinct enzymic constituent of succinoxidase (Clark, Neufeld, Widmer & Stotz, 1954) or rather a functional group of succinic dehydrogenase (cf. Green & Beinert, 1955), which in the presence of adequate hydrogen acceptors could oxidize succinate notwithstanding the inactivation of the BAL-sensitive factor. According to Slater (1949c), succinate and competitive inhibitors of succinic dehydrogenase prevent the effect of BAL on succinoxidase. This remote action of succinate and analogues is of considerable interest, as the 'protection phenomena' would no longer prove that there are thiols in the active centre of succinic dehydrogenase, as suggested by Hopkins *et al.* (1938) and others. On this ground the action of fluoride and phosphate on the inactivation of succinoxidase with BAL seemed worth trying. As shown in Table 7 the association of fluoride and phosphate does not modify the effect of BAL. Experiment A is particularly significant, as the partial inactivation of the enzyme rules out the

possibility that too long oxidation with BAL had nullified a difference due to the presence of fluoride. The slight protection of succinoxidase shown in that experiment is negligible if it is remembered that the same mixture of phosphate and fluoride prevents completely succinic-dehydrogenase inactivation by *o*-iodosobenzoate and other thiol reagents.

Action of fluoride on succinic dehydrogenase in the presence of ethylenediaminetetraacetate ions. Besides its action on succinic dehydrogenase, quoted above, phosphate strongly activates the oxidation of succinate by molecular oxygen, as it apparently promotes a closer association of succinic dehydrogenase to the cytochrome *c*-cytochrome oxidase system (Bonner, 1954). Ethylenediaminetetraacetate has a similar activating action on succinoxidase, though in much smaller concentrations (Bonner, 1954). The functional analogy between ethylenediaminetetraacetate and phosphate does not extend, however, to the promotion by the latter of succinic-dehydrogenase protection by fluoride, as shown in Table 8. If this related to the lack of action of fluoride on succinoxidase oxidation with BAL and the prevention of the effect of BAL by ethylenediaminetetraacetate (Stoppani & Brignone, in preparation), it may be concluded that agents able to control the activity of succinic dehydrogenase, on the one hand, and the mutual accessibility of succinoxidase components, on the other, have very

Table 7. *Inactivation of succinoxidase with BAL in the presence of fluoride and phosphate*

Succinoxidase (1 ml.) was oxidized with 8.0 mM BAL and additions as shown in Warburg flasks at 37° for 27 min. (Expt. A) or 25 min. (Expt. B) and then diluted with 10 ml. of distilled water (Expt. A) or 5.0 ml. 0.08 M phosphate buffer, pH 7.3 (Expt. B). Succinoxidase activity was measured in 0.4 ml. (Expt. A) or 0.3 ml. (Expt. B) of extract.

Expt.	Succinoxidase (mg.)	Additions	Oxygen taken up in BAL oxidation (μ l.)	Succinoxidase inhibition (%)
A	36.0	0.080 M phosphate	72	39.8
	36.0	0.080 M phosphate + 4.0 mM fluoride	91	36.2
B	28.4	0.080 M phosphate	101	91.5
	28.4	0.080 M phosphate + 4.0 mM fluoride	108	96.1

Table 8. *Effect of fluoride on succinic-dehydrogenase inactivation in the presence of ethylenediaminetetraacetate*

Succinoxidase (24.4 mg. in 1.0 ml.), was treated with 0.43 mM *o*-iodosobenzoate and additions as shown for 30 min. at 37° and then diluted with 4.0 ml. of distilled water. Succinic-dehydrogenase activity was measured in 0.3 ml. of this extract. Residual phosphate concentration in the initial enzyme suspension, 0.2 mM.

Additions	Succinic-dehydrogenase	
	Inhibition (%)	Protection (%)
None	91.8	—
1.95 mM ethylenediaminetetraacetate	88.5	3.6
4.0 mM fluoride	92.5	-0.7
1.95 mM ethylenediaminetetraacetate + 4.0 mM fluoride	83.5	9.0

different sites of action. Phosphate may be singled out as an exception, as it can affect the succinoxidase components in several ways which are not necessarily interdependent.

DISCUSSION

The protection of thiol groups of succinic dehydrogenase by fluoride in the presence of phosphate, in proportion to the concentration of both phosphate and fluoride, is a good confirmation of the mechanism proposed by Slater & Bonner (1952) to explain inhibition of succinic dehydrogenase by fluoride.

Succinate and competitive inhibitors of succinic dehydrogenase, on the one hand, and sulphhydryl reagents, on the other, are mutually exclusive with regard to succinic dehydrogenase. Since the work of Hopkins *et al.* (1938), this has been taken as evidence for location of the enzyme thiol on the active area of the enzyme, and deductions made from molecular models have led Potter & DuBois (1943) to postulate that the thiol is placed between two $-NH-$ groups with affinity for the carboxylate ions of succinate and some analogues. After binding the enzyme, thiol reagents hinder the access of the substrate to the affinity points and produce inhibition, and the enzyme-bound substrate prevents sulphhydryl reagents from reaching the thiols. Slater's (1949c) claim that 'protection phenomena' have no topical value for the area of succinoxidase shielded by succinate and competitive inhibitors is not substantiated by our observations with fluoride and similar ones with suramin (Stoppa & Brignone, in preparation). The possibility that a comparatively smaller affinity of these protectors for succinic dehydrogenase prevents their showing their action on the BAL-sensitive group can be ruled out, as the dissociation constants of the fluoride-(enzyme-phosphate), phosphate-(enzyme-fluoride) and suramin-enzyme complexes are respectively 6.7×10^{-5} and 3.3×10^{-4} (Slater & Bonner, 1952) and approx. 10^{-6} (Stoppa & Brignone, in preparation), i.e. about or below the values for the dissociation constants of the complexes of the enzyme with succinate and malonate (cf. Slater & Bonner, 1952; Thorn, 1953) which are supposed to prevent the enzyme oxidation with BAL (Slater, 1949c).

The remarkable specificity of fluoride and the requirement of bivalent ions, in particular phosphate, is difficult to explain without a better knowledge of the structure of succinic dehydrogenase. The same arguments considered by Slater & Bonner (1952) in relation to the enzyme inhibition by these ions still apply. The presence of haematin and non-haematin iron in some purified preparations of succinic dehydrogenase reported recently by Green, Kohout & Mii (1954) and Singer & Kearney (1954)

suggests the possibility of the formation of complexes that could explain the binding of fluoride and phosphate by this enzyme.

SUMMARY

1. Fluoride and phosphate together decrease markedly the inactivation of succinic dehydrogenase by thiol reagents, such as arsenicals, *o*-iodosobenzoate, iodoacetate and *N*-ethylmaleimide.

2. Chloride, bromide, iodide and thiocyanate do not replace fluoride. Sulphate and arsenate can replace phosphate to some extent, but nitrate, chloride and ethylenediaminetetraacetate do not.

3. Fluoride and phosphate do not prevent inactivation of succinoxidase by 2:3-dimercapto-propanol (BAL).

4. Sulphate increases the inhibition of succinic dehydrogenase by fluoride, but has less effect than phosphate. Nitrate and chloride do not promote the inhibition.

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