THE SARCOSINE OXIDASE IN ADAPTED AND UNADAPTED CULTURES OF A STRAIN OF PSEUDOMONAS AERUGINOSA

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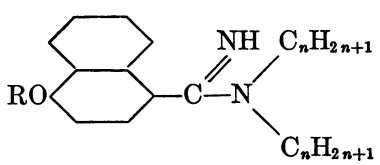
Kopper and Robin (1950) have shown that a strain of *Pseudomonas aeruginosa* that decomposes creatinine contains sarcosine oxidase, which oxidizes sarcosine to glycine and formaldehyde. The enzyme is present in the organism whether it is grown with creatinine or not and therefore can be classified as constitutive. Creatinine, however, causes an adaptive increase in enzyme concentration. Two questions of interest arise from these observations. The first is whether the increase in sarcosine oxidase concentration is accompanied by an increase in the ability of the adapted cell to oxidize glycine, formaldehyde, and formic acid, in other words, whether simultaneous adaptation occurs. The second is whether the properties of the adaptive and constitutive enzymes are the same. In the following it has been shown that there is no simultaneous adaptation and that, although the two enzymes are inhibited by the same drugs, the time characteristics of the inhibition indicate that the adaptive enzyme is on or near the cell surface whereas the constitutive one is in the interior.

EXPERIMENTAL RESULTS

Dr. Kopper kindly supplied the strain of *Pseudomonas*. It was grown for 24 hours in Difco nutrient broth, 8.0 g per liter with and without 5.0 g per liter of creatinine. The cells were centrifuged down and washed once with 50 ml of water. They were finally suspended in 0.05 M buffer phosphate, pH 7.8, so that the concentration of adapted and unadapted cells was the same. The autorespiration of each was small and served as a check on the number of cells in each suspension. The usual Warburg vessels were used; suitable concentrations of sarcosine, glycine, formaldehyde, and formate were added; and the rate of oxygen uptake was measured. The drugs used were a series of amidines of the general formula



where n varies from 2 to 6 and R is either a methyl or ethyl group. These compounds, which were kindly supplied by Dr. Richard Baltzly of the Wellcome Research Laboratories, inhibit the sarcosine oxidase of animal origin (unpublished results). The most effective is the C_6 compound; the C_2 is the least.

The length of the carbon chain is not the only factor, because if the ring with the methoxy group is substituted by a pyridine ring, no inhibition is apparent despite C_6 carbon chains. An ethoxy group on the ring is somewhat more effective than a methoxy group in compounds with the same carbon chains. On adapted cells equimolar concentrations of the various drugs caused the following percentage of inhibitions of the oxidation of sarcosine: C_6-methoxy, 90; C_5-methoxy, 31; C_5-ethoxy, 45; C_4-methoxy, 8; C_2-methoxy, 0; C_6-pyridine ring, 10.

TABLE 1

The effect of adding 6.2×10^{-4} M C_6-methoxy compound 5 minutes before and after 1.1×10^{-2} M sarcosine on the inhibition of the oxidation in the adapted and unadapted cells

(The figures are mm^3 O_2 uptake; the autorespiration has been subtracted; 37 C, pH 7.8.)

MIN	UNADAPTED			ADAPTED		
	Sarcosine	Sarcosine + drug first	Sarcosine + drug last	Sarcosine	Sarcosine + drug first	Sarcosine + drug last
20	26	22	25	104	59	100
40	60	46	58	233	131	210
60	97	71	93	356	198	342
80	146	103	135	497	278	472
100	195	136	177	592	344	580
120	255	174	225	639	408	636

TABLE 2

The effect of 6.2×10^{-4} M C_6-methoxy compound on the oxidation of 1.1×10^{-2} M sarcosine by adapted and unadapted cells

(The figures are mm^3 O_2 uptake; the autorespiration has been subtracted; 37 C, pH 7.8.)

MIN	UNADAPTED			ADAPTED		
	Sarcosine	Sarcosine + drug	% inhibition	Sarcosine	Sarcosine + drug	% inhibition
20	25	25	0	81	43	47
40	54	42	22	159	80	50
60	84	61	27	247	119	52
80	121	77	36	325	160	51
100	160	91	43	396	193	51
120	205	110	46	471	228	51
160	312	146	53	613	300	51

Most of the experiments were done with the C_6-methoxy compound. It was added to the bacteria 5 minutes before the sarcosine. If sarcosine is added first, the inhibition produced by the drug is much less. This is shown in table 1 and indicates that the inhibition is caused by the drug combining with the enzyme rather than by some nonspecific action.

Table 2 shows the typical effect of C_6-methoxy compound on the oxidation of sarcosine by unadapted and adapted cells. Despite the fact that the oxidation rate by the latter is more than twice as fast, the percentage of inhibition is

at first larger. Moreover, the inhibition reaches its maximum rapidly and thereafter remains fairly constant. In the unadapted cells the inhibition is small at the beginning and gradually increases. At the end of the experiment the inhibition may be equal or greater than it is in the adapted cells because less enzyme is present in the unadapted cells and consequently there are more drug molecules per enzyme molecules. The reasonable explanation for this effect is that the enzyme in the adapted cell is laid down on the surface and thus comes into immediate contact with the drug. The constitutive enzyme is in the interior so that time is required for the drug to penetrate to its site. To test this possibility further, a surface-acting detergent, sodium dodecyl sulfate, was added to both types of cells. The results are shown in table 3. The oxidation of the sarcosine by the adapted cell is inhibited whereas that by the unadapted cell is not. Apparently the dodecyl sulfate does not penetrate the cell wall.

TABLE 3

The effect of 0.5 mg per ml dodecyl sulfate on the oxidation of 1.1 × 10^{-2} M sarcosine by adapted and unadapted cells

(The figures are mm^3 O_2 uptake; the autorespiration has been subtracted; 37 C, pH 7.8.)

MIN	UNADAPTED			ADAPTED		
	Sarcosine	Sarcosine + drug	% inhibition	Sarcosine	Sarcosine + drug	% inhibition
20	22	25	0	51	35	32
40	52	53	0	103	71	32
60	87	84	2	157	109	31
85	139	134	4	229	156	32
105	193	178	8	285	192	32
125	248	227	9	335	229	32
165	381	341	10	432	292	32

The C_6-methoxy compound has little effect on the oxidation of formate and glycine in concentrations that inhibit the oxidation of sarcosine. When small inhibitions occur there is no difference between the two types of cells. Formaldehyde is also oxidized but it is toxic if more than 0.2 mg is added at one time. It is difficult to obtain accurate rates of oxidation, but the drug does inhibit significantly and to the same extent in both cell types. Apparently no adaptive enzymes are formed for the oxidation of glycine, formate, or formaldehyde since the ratio formate:sarcosine for the unadapted cells is 2.7, for the adapted, 0.73; glycine:sarcosine, 0.50 and 0.23, respectively; formaldehyde:sarcosine, 1.02 and 0.42, respectively.

Streptomycin and stilbamidine inhibit the oxidation of sarcosine in both cell types but only in relatively high concentrations.

SUMMARY

The sarcosine oxidase in a strain of *Pseudomonas aeruginosa* is increased by growing the organisms in media containing creatinine. There is no correspond-

ing increase in the enzymes that oxidize glycine, formaldehyde, and formic acid, the oxidation products of sarcosine.

Comparison of the actions of certain amidines and a detergent on the adapted and unadapted cells indicates that the extra sarcosine oxidase in the former is in or near the cell surface, whereas the constitutive enzyme is in the cell interior.

REFERENCES

KOPPER, P. H., AND ROBIN, L. 1950 The metabolic breakdown of sarcosine by a strain of *Pseudomonas aeruginosa*. Arch. Biochem., **26**, 458–460.