THE NATURE OF CARBONIC ANHYDRASE FROM PLANT SOURCES

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Summary

Carbonic anhydrase activity in leaves of plants is low compared with that in animal tissues.

In the absence of cysteine the activity of crude leaf extracts was readily lost.

A method of purification of the enzyme from crude leaf extracts is described. The most active preparation had a catalytic activity about 50-60 times that of the crude leaf extract.

During purification the enzyme was inactivated by dialysis in the absence of cysteine, by lead acetate, acetone, and by high concentrations of ammonium sulphate. The partially purified product contained 0.056 per cent. zinc, not removable by dialysis.

The enzyme is heat-labile.

The relation of activity to pH is described; optimum activity occurs within the range pH 6-8.

Activity of the enzyme preparation is inhibited only by relatively high concentrations of cyanide, sulphocyanide, azide, and sodium sulphide. Sulphanilamide in relatively high concentrations does not inhibit activity. Activity of the preparation is strongly inhibited by *p*-chlormercuribenzoate and by sodium arsenite; their inhibitory effects are reversible, reactivation occurring by the addition of simple thiol compounds such as cysteine and reduced glutathione.

Polarographic investigation suggests the presence of sulphydryl groups in the enzyme preparation.

The enzyme preparation from plant sources differs from that from animal sources in its inactivation during dialysis in the absence of cysteine, its inactivation by lead acetate, acetone, and ammonium sulphate, its relative insensitivity to inhibitors of metal proteins and to sulphanilamide, and in its strong and reversible inactivation by sulphydryl inhibitors.

It is suggested that carbonic anhydrase from plant sources is a different enzyme from that occurring in animal tissues.

I. INTRODUCTION

References to carbonic anhydrase activity in plants are few. Neish (1939) reported carbonic anhydrase activity in species of *Trifolium, Onoclea,* and *Arctium;* Day and Franklin (1946) found activity in leaves of some but not all species of *Sambucus* studied by them; Bradfield (1947) found activity in leaves of several species and investigated the effects of some inhibitors on the crude leaf extract.

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In this paper partial purification of the enzyme from leaves of spinach beet (*Beta vulgaris*) has been attempted and some properties of the product described.

II. Carbonic Anhydrase Activity in Crude Leaf Extracts

(*a*) *Measurement of Activity*

For measurement of activity a manometric method using the boat apparatus of Meldrum and Roughton (1934) was used; this depends upon the rate of evolution of CO_2 from sodium bicarbonate solution mixed with phosphate buffer at pH 6.8. In the arms of the flask were placed 2 ml. of 0.2M $NaHCO_3$ solution dissolved in 0.02M NaOH and 2 ml. of 0.2M mixed phosphate buffer to which was added 0.5 ml. of the plant extract. All estimations were carried out at 5°C.

The unit of activity adopted was the "enzyme unit" (E.U.) of Meldrum and Roughton, but measured at 5°C. instead of 15°C., and defined as the amount of enzyme preparation which, when dissolved in 4 ml. of phosphate-bicarbonate mixture, gives a value of $(R - R_0)/R_0$ equal to unity at 5°C., where R and R_0 are respectively the rates of reaction in the presence and absence of the enzyme.

(*b*) *Estimation of Zinc*

Zinc content of the preparation was determined polarographically by the method described by Wood and Sibly (1950).

(*c*) *Preparation of Extracts for Estimation of Activity*

Samples of leaves, each of 5 g. fresh weight, were ground finely in a mortar for 2 min. with acid-extracted sand together with 10 ml. freshly prepared cysteine-buffer solution. A 0.2M mixed phosphate buffer (pH 6.8) containing equimolecular amounts of KH_2PO_4 and Na_2HPO_4 was prepared on each occasion by diluting stock solutions of the salts of molar concentration with CO_2-free distilled water. The final buffer was prepared by mixing 2 parts of the 0.2M phosphate buffer with 3 parts of cysteine solution so that the final concentration of cysteine was 0.01M. The pH of this solution was 6.6. After grinding, the resultant brei was kept at 2°C. until estimations of activity had been performed.

In our experience any leaf extract, even at 2°C., without addition of cysteine during grinding lost its activity rapidly. (Table 1.)

Activity of the extract was also influenced by the method of disintegration of the leaf material. Disintegration for 2 min. in a Waring Blendor packed with ice reduced the activity of the brei to approximately one-half that obtained by grinding in a mortar for the same time: e.g. aliquots of material ground in the Waring Blendor had a range of activity at 5°C. from 10 to 15 enzyme units per ml., whilst the same number of aliquots ground with sand in a mortar gave a range of activities from 20 to 25 enzyme units per ml. at the same temperature.

These results after disintegration for 2 min. were consistently reproducible. Loss of activity, variable in amount, occurred when grinding in a mortar was prolonged, as for example when large amounts of leaf material were used. For this reason, when preparing large amounts of extract for purification of the enzyme, the material was disintegrated for 2 min. in a Waring Blendor cooled with ice. At the same time the loss of activity involved in this procedure was measured by comparing the activity of an appropriate aliquot of the extract with that of 5 g. fresh material ground in a mortar.

Extracts as prepared above were inactivated when dialysed against distilled water; it was found essential to dialyse the extract against dilute cysteine solution to avoid loss of activity.

TABLE 1

EFFECT OF 0.01M CYSTEINE ON ACTIVITY OF LEAF BREI STORED AT 2°C.

Time after Grinding (hr.)	Without Cysteine		With Cysteine	
	Activity (E.U./0.5 ml. extract)	Loss (%)	Activity (E.U./0.5 ml. extract)	Loss (%)
1	3.17	0	3.17	0
2	2.3	27	—	—
4	1.7	46	—	—
5	0.3	90	3.17	0
24	0	100	3.17	0

All measurements at 5°C.

III. PURIFICATION OF CARBONIC ANHYDRASE

A modification of the method of Keilin and Mann (1940) was used in purification of the enzyme.

Fresh leaves of spinach beet (*Beta vulgaris* var. *cicla*) were disintegrated for 2 min. with ice-cold 0.01M cysteine solution in a Waring Blendor cooled with ice and the mixture filtered through gauze to remove uncrushed debris. To this brei was added slowly sufficient 95 per cent. ethanol to bring the final concentration to 30 per cent., the temperature being kept near 0°C. To this mixture one-tenth of its volume of chloroform was added and the whole shaken for about 1 min. The mixture was then centrifuged at 1,700g for 10-15 min. This resulted in the formation of three layers, the lowest being chloroform, which contained all the chlorophyll and some fats; above this occurred a sludge of greenish white, coagulated cell constituents, partially protein in nature, and above this a yellow, translucent solution, which contained approximately 85 per cent. of the original enzyme activity. This solution — the "alcohol-chloroform extract" — when decanted was extremely stable, no loss of enzyme activity being observed after 27 days storage at 2°C. The stability of this enzyme preparation was in contrast with more highly purified preparations described later. Small amounts only were prepared on each occasion; in

general, 1 kg. leaf material and 850 ml. 0.01M cysteine solution were used, yielding about 800 ml. of leaf brei and about 1,150 ml. of "alcohol-chloroform extract."

Addition of lead acetate or of acetone to the "alcohol-chloroform extract" caused complete inactivation of carbonic anhydrase in the extract and for this reason these substances could not be used for purification (cf. Keilin and Mann 1940).

Willstätter's (1920) alumina Cγ cream was added slowly with stirring to the "alcohol-chloroform extract," the solution allowed to stand for 5-10 min. and then centrifuged at 1,700g for 10 min.

The amount of alumina cream must not be less than 800 mg. alumina/100 ml. extract. As shown in Figure 1, lower concentrations of alumina resulted in incomplete adsorption of enzyme. These results were obtained by adding varying amounts of alumina to 100 ml. of extract and measuring the activity of both supernatant solution and the eluate from the alumina precipitate. $Ca_3(PO_4)_2$ used as an adsorbing agent by Meldrum and Roughton (1934) and by Keilin and Mann (1940) was as effective as but no better than alumina Cγ; 3.0 g. $Ca_3(PO_4)_2$ per 100 ml. extract adsorbing the enzyme completely.

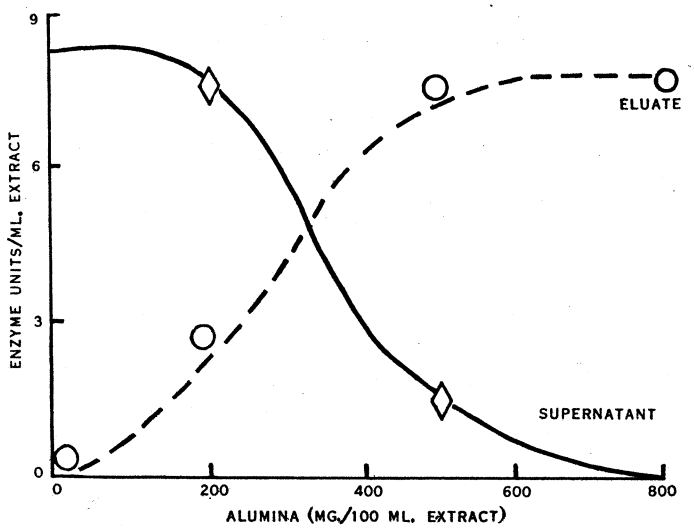

Fig. 1.—Carbonic anhydrase activity (E.U./ml. extract) absorbed by different amounts of alumina Cγ (mg./100 ml. extract).

Centrifugation caused the alumina to be concentrated into a cake, which after discarding the supernatant solution, was broken up and washed two or three times with 0.1M Na_2HPO_4 to elute the enzyme. Approximately 40 ml. of phosphate solution were required for each litre of original "alcohol-chloroform extract."

To the combined eluates sufficient saturated ammonium sulphate solution was added to give a 50 per cent. saturated solution. After standing for 2 hr.

at 2°C. the precipitate was centrifuged off and dried in a vacuum desiccator. Further addition of ammonium sulphate to give 100 per cent. saturated solution brought down another precipitate which was dried in a similar manner but contained only very small activity. The solution after precipitation possessed no carbonic anhydrase activity.

The dried precipitate from 50 per cent. saturated ammonium sulphate lost its activity slowly after the first 2 or 3 days even in a vacuum desiccator (see Table 2). Activities of the precipitates from 50 per cent. saturated ammonium sulphate were consistently reproducible with different batches of leaf material but further purification has not been successful.

TABLE 2

LOSS OF CARBONIC ANHYDRASE ACTIVITY OF DRIED PARTIALLY PURIFIED ENZYME IN A VACUUM DESICCATOR AT 15°C.

Days after Preparation	Activity (E.U./mg.)	Mean Loss (%)
1	2.88	0
2	2.88	0
3	2.0	30
6	1.1	62
7	0.66	70

Conditions of estimation: 4 mg. enzyme preparation in 2 ml. 0.2M phosphate buffer at pH 6.8; temperature of estimation, 5°C.

The precipitate obtained by 50 per cent. saturation with ammonium sulphate was dissolved and dialysed against distilled water until free from salt (48 hr.). Dialysis against distilled water caused a loss of 75 per cent. of weight of the original precipitate material but in all cases caused complete inactivation of the enzyme. Dialysis against 0.01M cysteine solution caused a similar reduction in weight and a marked, though variable, decrease in activation of the enzyme. The fragility of carbonic anhydrase from plant sources compared with that from animals will be discussed later. In calculating the activity of the material precipitated by ammonium sulphate in Table 3, measurements were taken before dialysis and activity expressed on the basis of weight of product after dialysis.

The enzyme was inactivated only in crude leaf extract in the absence of cysteine; at later stages of purification, including the final product, the same activity was obtained in the presence and absence of cysteine.

In Table 3 the increase in activity of the preparations at various stages of purity is shown and, for comparison, figures obtained by Keilin and Mann (1940) for analogous stages in purification of the enzyme from animal sources.

IV. Zinc Content

At all stages the preparations contained zinc. The mean content of the dried preparation after precipitation with 50 per cent. ammonium sulphate was 0.014 per cent. zinc. On the basis of dry weight after dialysis this is equivalent to 0.056 per cent. zinc.

V. Properties of the Partially Purified Enzyme

(*a*) *Temperature*

The enzyme is heat-sensitive. Complete inactivation of the 50 per cent. $(NH_4)_2SO_4$ precipitate in phosphate buffer of pH 6.8 resulted in 5 min. at 55°C., without obvious coagulation, and 5 min. at 50°C. caused 85 per cent. inactivation.

Table 3

CARBONIC ANHYDRASE ACTIVITIES OF PREPARATIONS AT VARIOUS STAGES OF PURITY

	Plant Source Activity			Animal Source Activity* 15°C.
	5°C.	15°C.		A†
Plant extract	0.58	0.29	Erythrocytes	14.3
"Alcohol-chloroform extract"	0.80	0.40	"Alcohol-chloroform extract"	133
Phosphate eluent	33	18		
Purest $(NH_4)_2SO_4$ ppt.			Purest final product	2,200
50% saturated	10	6		
100% saturated	0.5	—		

* Activities in E.U./mg. dry wt. of preparation. Temperatures given are those at which estimations were carried out.

† Erythrocytes from defibrinated ox blood. Data of *A* from Keilin and Mann (1940).

(*b*) *Activity in Relation to pH*

At all stages of preparation the pH was within the range 5.8-7.0. Beyond these limits activity was lost rapidly. Samples of the enzyme preparation precipitated with 50 per cent. ammonium sulphate were dissolved in 0.3 ml. of buffer solutions in the reaction flasks and stood for 30 min. at 15°C. The activity was then determined at 5°C.

The pH, composition, and concentrations of the buffers used were as follows: pH 1.04, 0.1M HCl; pH 1.55, 0.023M citric acid *plus* 0.046M NaOH *plus* 0.2M HCl; pH 4.96, 0.1M citric acid *plus* 0.2M NaOH; pH 6.60, 0.086M Na_2HPO_4 *plus* 0.114M KH_2PO_4; pH 8.05, 0.0045M NaOH *plus* 0.05M H_3BO_3 *plus* 0.05M KCl; pH 9.30, 0.0294M NaOH *plus* 0.05M H_3BO_3 *plus* 0.05M KCl; pH 11.9, 0.1M NaOH. Activities in relation to pH are shown in Table 4.

(*c*) *Effect of Inhibitors*

The effects of various inhibitors on carbonic anhydrase activity are described below. In all cases the enzyme preparation used was that precipitated by 50 per cent. $(NH_4)_2SO_4$: 4 mg. of this preparation was dissolved in 2 ml. 0.2M phosphate buffer of pH 6.8. To this solution 0.2 ml. of a solution containing the inhibitor was added, both solutions being kept at 5°C. The solu-

tion of inhibitor contained sufficient of the latter to give the final concentrations indicated below. Estimations of enzyme activity were made at 5°C. 2-3 min. after addition of the inhibitor.

General.—General enzyme poisons inhibited the activity of carbonic anhydrase. Mercuric chloride caused 100 per cent. inactivation in 1.0×10^{-4}M solution. Merthiolate (sodium ethyl mercurithiosalicylate) gave 100 per cent. inhibition with 5.0×10^{-5}M solution.

Metal-protein inhibitors.—Both KCN and KCNS caused inhibition to approximately the same extent, 1.0×10^{-3}M solutions causing practically no inhibition whilst 1.0×10^{-2}M solutions caused about 65 per cent. inhibition.

NaN_3 was more effective as an inhibitor than KCN. 1.0×10^{-2}M solution caused 100 per cent. inhibition and 1.0×10^{-3}M solution 70 per cent. inhibition.

With Na_2S, a 1.0×10^{-3}M solution caused about 5 per cent. inhibition whilst 1.0×10^{-2}M caused 70 per cent. inhibition.

TABLE 4

ACTIVITY OF CARBONIC ANHYDRASE AFTER 30 MIN. AT 15°C. AT THE pH INDICATED

Buffer	pH	Activity (as % of activity at pH 6.6)
HCl	1.04	12
Citrate — HCl	1.55	27
Citrate — HCl	4.96	27
Phosphate	6.60	100
NaOH-borate	8.05	100
NaOH-borate	9.30	35
NaOH	11.90	12

Activity measured at 5°C. Ionic concentrations of buffers used are indicated in the text.

Sulphonamides.—Sulphanilamide solution at 1.0×10^{-3}M caused no inhibition and a 1.0×10^{-2}M solution less than 5 per cent. inhibition.

Sulphydryl inhibitors.—In view of the role of cysteine in preserving activity of crude enzyme solutions the effect of various sulphydryl inhibitors was investigated.

Sodium iodoacetate caused no inhibition in 1.8×10^{-2}M solution. However, this compound, an alkylating agent, does not necessarily react with all -SH groups present and often reacts with amino groups.

Mercaptide-forming compounds (e.g. *p*-chlormercuribenzoate and arsenicals) have a much greater affinity for -SH groups, combining with the protein to give reversible compounds that dissociate readily on addition of another thiol compound with greater affinity for the reagent, e.g. cysteine or reduced glutathione. The degree of reactivation of an enzyme inhibited by a mercaptide-forming reagent is determined mainly by the affinity of the enzyme and the thiol for the inhibitor. Generally, the higher the concentration of the thiol compound the greater the degree of reactivation.

p-Chlormercuribenzoate caused complete inhibition in 1.0×10^{-4}M solution and inhibiting effects were apparent even at dilutions of 2.2×10^{-6}M (Table 5).

TABLE 5

INHIBITION OF CARBONIC ANHYDRASE ACTIVITY BY *p*-CHLORMERCURIBENZOATE

Concentration of Inhibitor	Percentage Inhibition
1.0×10^{-4}M	100
4.3×10^{-5}M	97
2.2×10^{-5}M	90
4.3×10^{-6}M	40
2.2×10^{-6}M	24

Conditions of experiment: 4 mg. enzyme preparation in 0.2M phosphate buffer at pH 6.8; estimations made 2 min. after addition of inhibitor; temperature 5°C.

Reactivation of the poisoned enzyme by reduced glutathione is shown in Table 6, addition of the glutathione taking place 2-3 min. after addition of the *p*-chlormercuribenzoate to the enzyme solution.

TABLE 6

REACTIVATION OF *p*-CHLORMERCURIBENZOATE-INHIBITED CARBONIC ANHYDRASE BY GLUTATHIONE

Concentration of *p*-Chlormercuribenzoate	Percentage Inhibition	Concentration of Glutathione	Ratio $\frac{\text{Conc. Reactivator}}{\text{Conc. Inhibitor}}$	Percentage Reactivation
1.0×10^{-4}M	100	1.6×10^{-3}M	16	0
4.3×10^{-5}M	100	1.6×10^{-3}M	37	84
2.2×10^{-5}M	87	1.6×10^{-3}M	73	100

Conditions of experiment: 4 mg. enzyme preparation in 2 ml. 0.2M phosphate buffer at pH 6.8; estimations of inhibition made 2 min. after addition of inhibitor; glutathione added 2 min. after addition of inhibitor; temperature 5°C.

The effect of sodium arsenite on inhibition of carbonic anhydrase was similar to that of *p*-chlormercuribenzoate. 100 per cent. inhibition was obtained by 5×10^{-3}M solution of arsenite. Cysteine at 5×10^{-2}M gave 20 per cent. reactivation and 1.0×10^{-1}M cysteine gave 95-100 per cent. reactivation of the arsenite-poisoned enzyme.

VI. SULPHYDRYL GROUPS IN THE PARTIALLY PURIFIED ENZYME

Carruthers (1947) developed a polarographic method for estimating the amounts of certain proteins containing -SH groups (e.g. cytochrome *c*, ascorbic acid oxidase, tyrosinase) by the catalytic wave they developed in a solution of ammonium chloride, ammonium hydroxide, and hexamino-cobaltic chloride. We confirmed that only amino acids containing -SH groups gave this wave. The partially purified products used by us, prepared with and without cysteine in the initial treatment, all gave a wave similar to that for cysteine, gluta-

thione, and cytochrome *c* (Fig. 2). We concluded, therefore, that our product contains sulphydryl groups.

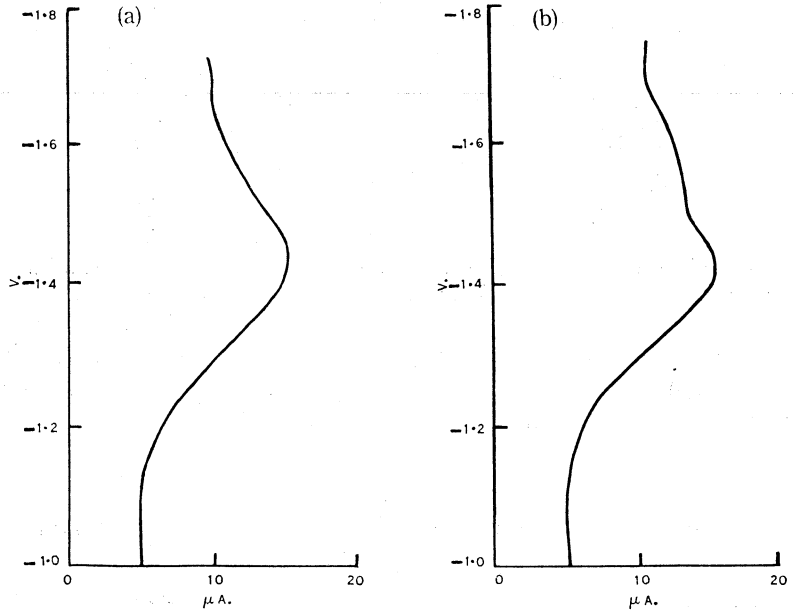

Fig. 2.—Polarographic tracing (V./μA.) of (*a*) cytochrome *c* (10 g./ml.) and (*b*) carbonic anhydrase preparation (0.1 mg./ml.). The similarity of the tracings is due to the presence of sulphydryl groups.

VII. Discussion

The stability of carbonic anhydrase in crude leaf extracts is dependent upon the presence of certain reducing substances. Day and Franklin (1946) found that activity of such extracts was quickly lost but was retained in part by addition of stannous chloride. Bradfield (1947) used cysteine instead of stannous chloride to preserve activity. This effect of cysteine in preserving activity in crude leaf extracts has been confirmed by us and rate of loss of activity in its absence measured.

Apparently the presence of a reducing substance is necessary only in crude leaf extracts. The enzyme is stable in the "alcohol-chloroform extract" and in the phosphate eluate from alumina in the absence of cysteine, and activities of these solutions are the same whether measured in the presence or absence of cysteine. The enzyme preparation obtained by precipitation with 50 per cent. ammonium sulphate is more stable than the crude leaf extract but less stable than the enzyme in the two foregoing extracts; the activity of this preparation is not affected by the presence of cysteine. As described below carbonic anhydrase probably contains sulphydryl groups; cysteine and stannous chloride apparently protect the enzyme from oxidation by enzymes present in the crude leaf extract.

The amount of enzyme in leaf tissues is small compared with that in animal tissues (cf. Table 3). If the most active preparations obtained during purification of extracts from plant and animal sources are compared (the phosphate eluate from plant extract and Keilin and Mann's purest preparation) the increase in activity relative to that of the initial extracts is of approximately the same order in both cases viz. 50-60 times in plant material and 60-150 times in Keilin and Mann's preparation. However, activities (E.U./mg.) of the plant preparations are very low compared with those from animal sources at all stages of purification.

Purification of the plant enzyme is more difficult than that from animals, not only because of its low concentration in the tissues, but also because of its greater fragility.

Inactivation of carbonic anhydrase in crude leaf extract occurs in the absence of cysteine and during dialysis against distilled water. In the "alcohol-chloroform extract" complete inactivation is brought about by addition of lead acetate and of acetone. Furthermore, it may be seen from Table 3 that addition of 50 per cent. saturated $(NH_4)_2SO_4$ to the solution eluted by phosphate from alumina caused marked reduction in activity. Investigation showed that addition of 0.15M $(NH_4)_2SO_4$ to the "alcohol-chloroform extract" caused 15 per cent. inhibition of the original activity. The precipitate produced by addition of 50 per cent. $(NH_4)_2SO_4$ was inactivated by dialysis.

The procedures mentioned in the last paragraph do not decrease activity of the purified enzyme from animal sources. These differences in behaviour suggest the possibility that the enzyme from plant sources may be different from that from animal sources.

This possibility becomes more definite when the effects of inhibitors on enzymes from the two sources are compared. In our partially purified preparations from plants, KCN, KCNS, and Na_2S caused practically no inhibition in 1.0×10^{-3}M solutions, and about 65 per cent. inhibition in 1.0×10^{-2}M solutions. NaN_3 was more effective as an inhibitor than the foregoing compounds; 1.0×10^{-2}M solution caused 100 per cent. inhibition and 1.0×10^{-3}M solution 70 per cent. inhibition. Bradfield (1947) also reported that inactivation of the enzyme in crude leaf extracts was brought about only by high concentrations of cyanide and azide.

It is apparent that these substances are less effective as inhibitors of carbonic anhydrase from plant sources than is the case with the animal enzyme. Meldrum and Roughton (1934) found that 1.25×10^{-3}M KCN caused complete inhibition of their partially purified enzyme and also of that in crude ox blood. Keilin and Mann (1940) found that concentration of KCN as low as 4.0×10^{-6}M caused 85 per cent. inhibition of activity of their purified enzyme.

Even more marked is the contrast in behaviour of carbonic anhydrase from plant and animal sources towards sulphanilamide. Bradfield (1947) stated that sulphanilamide was ineffective in causing inactivation of the enzyme in crude leaf extracts. Sulphanilamide in 1.0×10^{-3}M solution caused no inactivation and 1.0×10^{-2}M solution less than 5 per cent. inhibition of original

activity of our partially purified product. With carbonic anhydrase from animal sources Mann and Keilin (1940) showed that the enzyme was strongly inhibited by sulphanilamide which they claim is highly specific for animal carbonic anhydrase. They showed that concentrations of 2×10^{-5}M sulphanilamide caused almost 100 per cent. inhibition of activity and concentrations as low as 2×10^{-6}M more than 50 per cent. inhibition of the original activity.

In view of the relative insensitivity of the plant enzyme to the foregoing inhibitors the possibility that the plant enzyme is not a metal-protein cannot be overlooked. However, this insensitivity could have been caused by the inhibitors combining with impurities (e.g. heavy metals) more readily than with the enzyme. Polarograms showed the presence of copper, as well as zinc, in our preparations and it is possible that other heavy metals were also present.

Wood and Sibly (1950) showed that zinc was not removed from crude plant extracts of oat leaves by dialysis against distilled water. All our preparations contained zinc, the precipitate with 50 per cent. ammonium sulphate contained 0.014 per cent. zinc, and the inactive product after dialysis 0.056 per cent. zinc. The purest preparation of Keilin and Mann from animal sources contained 0.31-0.33 per cent. zinc. In the absence of criteria that our preparation was a homogeneous protein, comparison with regard to zinc content between the two enzyme preparations cannot be made, nor a decision reached whether zinc is an essential constituent of the enzyme.

Since cysteine and stannous chloride preserve carbonic anhydrase activity in crude leaf extracts, Bradfield (1947) suggested that the plant enzyme shows greater dependence on sulphydryl groups than does the animal enzyme. The marked inhibition of activity of our enzyme preparation caused by low concentrations of mercaptide-forming compounds such as *p*-chlormercuribenzoate and sodium arsenite, and the reversal of these inhibitions by thiol compounds are evidence for the presence of sulphydryl groups in the enzyme. This view is supported by polarographic evidence for the presence of such groups in our partially purified product. We know of no evidence that carbonic anhydrase from animal sources is inhibited by mercaptide-forming compounds.

In view of the marked differences in behaviour of the enzyme preparation from plant tissues compared with that from animal sources it is suggested that plant carbonic anhydrase is a different enzyme from that occurring in animal tissues.

VIII. References

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