SPECIAL ARTICLES

AZIDE INHIBITION OF ANAEROBIC ASSIMILATION OF GLUCOSE BY YEAST AND ITS APPLICATION TO THE DETERMINATION OF FERMENTABLE SUGAR

IN the determination of fermentable sugar by yeast fermentation methods, the amount of carbon dioxide produced is usually 10 to 35 per cent. lower than that expected from the familiar equation for alcoholic fermentation. The low recoveries appear to be due to the assimilation of a portion of the sugar to form intracellular carbohydrate, since anaerobic assimilation of appreciable amounts of glucose by yeast has been observed even in very short term experiments.[1, 2]

Sodium azide and other respiratory poisons have been observed to inhibit the anaerobic uptake of ammonia[3] and the aerobic assimilation of several oxidizable substrates[4, 5, 6] by yeast. This suggested that the anaerobic assimilation of fermentable sugars could also be stopped by using these inhibitors, thus allowing conversion of all the fermentable sugar to carbon dioxide and alcohol. This possibility was tested by measuring manometrically the total carbon dioxide produced from the anaerobic fermentation of known amounts of glucose by washed bakers' yeast in the presence or absence of 10^{-3} M sodium azide. Table 1 shows typical results. The total carbon dioxide production corresponded to 67 per cent. of the added glucose in the absence of inhibitor, and 100 per cent. in the presence of 10^{-3} M sodium azide. The rate of fermentation was not altered by this concentration of azide. Table 1 also shows a similar increase in the carbon dioxide produced from the fermentable sugar in rat serum and in tungstic acid filtrates of this serum by yeast when azide was present. Known amounts of glucose were completely fermented in rat serum in the presence but not in the absence of azide.

TABLE 1

EFFECT OF 10^{-3} M SODIUM AZIDE ON RECOVERY OF GLUCOSE (TEMPERATURE 30° C., PH 4.5, 0.05 M SUCCINATE, 25 MG WASHED BAKERS' YEAST IN NITROGEN GAS)

Azide	Glucose	Rat serum	Tungstic acid filtrate of rat serum (1:10)	Total CO_2 produced	Glucose equivalent to CO_2	Per cent. recovery
m	*mg*	*cc*	*cc*	*mm*³	*mg*	
0	0	0	0	7.0	0.028	..
0	1.0	0	0	166	0.67	67
10^{-3}	1.0	0	0	251	1.01	101
0	2.0	0	0	336	1.35	67
10^{-3}	2.0	0	0	505	2.04	102
0	0	.3	0	87	0.35	..
10^{-3}	0	.3	0	128	0.52	..
10^{-3}	1.0	.3	0	382	1.54	102
0	0	0	1.5	44	0.18	..
10^{-3}	0	0	1.5	64.0	0.26	..
10^{-3}	1.0	0	1.5	320	1.29	103

If young (24-hour) pure cultures of yeast (*Saccharomyces cerevisiae* isolated from commercial bakers' yeast) were used immediately upon removal from slants and washing by centrifugation, glucose recoveries were only 5 to 10 per cent. lower than theoretical. If however, the yeast was aerated 2 to 10 hours after washing, the recoveries were increasingly low, reaching a minimum of about 33 per cent. lower than theoretical. This value of 67 per cent. recovery was obtained repeatedly in aerated yeast, and suggests that under optimum conditions for assimilation, one third of the glucose is synthesized into cell material (presumably glycogen), the rest being fermented. Similar results have been obtained in the aerobic metabolism of glucose and other substrates.[4, 5, 6, 7]

The fact that anaerobic assimilation of glucose and ammonia can be completely inhibited by cellular poisons usually considered to be inhibitors of heavy metal enzymes might suggest that iron-containing enzymes are involved in the assimilation of both carbon and nitrogen by yeast. However, a selective inhibition of aerobic assimilation has been described for a number of other toxic substances including iodoacetate and dinitrophenol, and the azide inhibition of anaerobic assimilation should not yet be considered good evidence that heavy metal catalysts are involved in the assimilation reactions.

These results indicate that an improvement in the manometric or titrometric determination of fermentable carbohydrate may be achieved by carrying out the yeast fermentation in the presence of sodium azide. We have found it satisfactory to place the sample containing 0.2 to 2 mg of glucose equivalent buffered at pH 4.5 with 0.05 M succinate and 10^{-3} M in sodium azide in the main compartment of Warburg vessels. About 25 mg of washed bakers' yeast is added from the side arms after temperature equilibration and removal of oxygen with nitrogen. The production of carbon dioxide is followed until it virtually ceases (in 10 to 40 minutes), and the amount of fermentable car-

[1] C. B. van Niel and E. H. Anderson, *Jour. Cell. and Comp. Physiol.*, 17: 49, 1941.

[2] R. J. Winzler and J. P. Baumberger, *Jour. Cell. and Comp. Physiol.*, 12: 183, 1938.

[3] R. J. Winzler, Dean Burk and V. du Vigneaud (in press).

[4] C. E. Clifton, *Enzymologia*, 4: 246, 1937.

[5] R. J. Winzler, *Jour. Cell. and Comp. Physiol.*, 15: 343–354, 1940.

[6] M. J. Pickett and C. E. Clifton, *Jour. Cell. and Comp. Physiol.*, 22: 147, 1943.

[7] C. B. van Niel and A. L. Cohen, *Jour. Cell. and Comp. Physiol.*, 20: 95, 1942.

bohydrate is then calculated as glucose from the following relation:

$$\text{mg. of glucose in sample} = \frac{\text{mm}^3\ CO_2\ \text{produced}}{248\ \text{mm}^3}$$

No determination of a correction factor from standard glucose solutions is necessary.

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CONVERSION OF GLOBULAR TO ORIENTED FIBROUS PROTEINS

IT was reported recently[1] that several globular proteins can be changed into a fibrous form by heating them in the presence of water and subjecting them to high shear stress. As evidenced by x-ray diffraction, the converted protein molecules have substantially the same spatial arrangement as the molecules of the natural fibrous protein, β-keratin, as it occurs in feathers and stretched wool and hair. To the list of globular proteins that can thus be made fibrous in the molecular sense may now be added gliadin, the mixed proteins of blood serum[2] and the globulins of tobacco and pumpkin seed.[2]

Conversion from the globular to the fibrous form has also been effected by soaking protein filaments in aqueous solutions of various reagents, followed by stretching, both treatments being carried out at room temperature.[3,4] Ovalbumin, lactoglobulin, casein and pumpkin seed globulin have been converted from the globular to the oriented fibrous form in this way. Ovalbumin has been particularly easy to unfold and orient. β-keratin diffraction patterns have been given by ovalbumin filaments stretched at room temperature after being treated with such aqueous solutions as the following: 75 per cent. methanol, 75 per cent. ethanol, 75 per cent. isopropanol, 50 per cent. t-butanol, saturated benzyl alcohol, 5 per cent. phenol, 75 per cent. formamide, 50 per cent. urethane, 50 per cent. pyridine, saturated aniline, 75 per cent. acetaldehyde, 90 per cent. acetone, saturated methyl ethyl ketone, 50 per cent. ethylene glycol monoethyl ether, 75 per cent. dioxane, 10 per cent. chloral hydrate, 10 per cent. silver nitrate, saturated cerous nitrate, 50 per cent. cupric nitrate, 10 per cent. trichloroacetic acid, 25 per cent. sulfuric acid, 20 per cent. hydrochloric acid, 17 per cent. nitric acid, 10 per cent. toluene sulfonic acid and 5 per cent. sodium hydroxide. Ovalbumin filaments soaked in acetic anhydride, glacial acetic acid, 98–100 per cent. formic acid, 65 per cent. lithium bromide, 50 per cent. ammonium thiocyanate, or saturated aqueous solutions of either urea or guanidine hydrochloride, and then in water were stretched to give the β-keratin structure.

It should be mentioned that the "egg-white" pattern of Astbury, Dickinson and Bailey,[3] characterized by the 9.8 Å reflection on the equator and the 4.7 Å reflection on the meridian, has been obtained also from chemically treated ovalbumin.[1]

The concentration of the reagent is not critical, and the time of soaking required to render the filaments stretchable ranges from a few minutes to several hours. For example, filaments soaked in saturated ammonium thiocyanate for two minutes and then in water for three minutes were stretched to a draw ratio (ratio of final to initial length) of 4.9 and gave the β-keratin pattern. Soaking in saturated aniline did not produce good stretching characteristics until after about five hours. Roughly, a draw ratio of five is required to give good orientation. If internal friction is small, the high shear required for orientation is not developed on stretching, and despite a large draw ratio the specimen gives only an amorphous diffraction pattern. Conversely, if cohesion and internal friction are large, a draw ratio of only two or three will give good fiber patterns. Ordinarily, filaments are most easily stretched while moist, but certain ovalbumin preparations, particularly those treated with phenol solution, may be stretched readily when air-dry. The phenol-treated ovalbumin filaments were also remarkable in that they exhibited the typical characteristics[5] of cold drawing, that is, on application of tensile stress they "necked down" and with continued stretching the necked-down, oriented section grew at the expense of the larger, unoriented sections.

Chemical treatment increases the degree of ordering of the peptide chains. Strong diffraction rings at approximately 4.7 and 9.8 Å sharpen, and fainter rings are resolved from diffuse halos.[6] As many as five diffraction rings have been obtained from ovalbumin treated with aqueous methanol, formamide or urethane. The rings from the formamide-treated ovalbumin occurred at 10.7, 4.7, 3.7, 2.2 and 2.0 Å. As a rule, preparations showing the largest number of diffraction rings and the sharpest rings were most easily oriented and gave fiber patterns containing the greatest

[1] F. R. Senti, C. R. Eddy and G. C. Nutting, *Jour. Am. Chem. Soc.*, 65: 2473, 1943.

[2] We should like to thank Sharp and Dohme, Inc., for the serum proteins, and H. B. Vickery, of the Connecticut Agricultural Experiment Station, for the pumpkin seed globulin.

[3] W. T. Astbury, S. Dickinson and K. Bailey, *Biochem. Jour.*, 29: 2351, 1935.

[4] K. J. Palmer and J. A. Galvin, *Jour. Am. Chem. Soc.*, 65: 2187, 1943.

[5] W. H. Carothers and J. W. Hill, *Jour. Am. Chem. Soc.*, 54: 1579, 1932.

[6] G. L. Clark and J. H. Shenk, *Radiology*, 28: 58, 144, 1937, observed sharpening of the 4.7 and 9.8 Å diffraction rings of ovalbumin and hemoglobin precipitated from dilute solution by trichloroacetic acid, ethanol, acetone or formalin. M. Spiegel-Adolph and G. C. Henny, *Jour. Phys. Chem.*, 46: 58, 1942, observed sharpening of the pattern of pseudoglobulin denatured by ethanol.