per cent only of the total nitrogen went into solution which was colourless indicating the absence of soluble quinonoid material. Complete solution of powdered ootheca was obtained in conc. HCl at 37° in 4 days. Removal of the HCl below 37° and digestion of the residue with papain[10] gave a solution in which protocatechuic acid was the only ether-soluble phenol.

Fusion of powdered, fully hardened oothecae (2 g) with KOH (20 g) and water (10 ml) at 270–280° for 2 h in an atmosphere of nitrogen with stirring gave *p*-hydroxybenzoic acid (approx. yield 3.6 %). This acid was identified in the ethereal extract of the acidified aqueous solution of the melt by the use of paper chromatography[11]. Several other phenols were present in extremely small amounts. The *p*-hydroxybenzoic acid was probably formed from tyrosine.

Amino phenols could not be detected in hydrolysates of the oothecae. Protocatechuic acid and tyrosine were the only phenolic compounds identified. Of these protocatechuic acid is present uncombined in the original material. Exhaustive extraction of the powdered, fully hardened oothecae with hot water yielded 1 % of protocatechuic acid (identified as above) which is more than sufficient to account for the amounts of this acid identified in the hydrolysates. For these experiments the oothecae were cleaned by washing in water[7] and no doubt some protocatechuic acid was lost in the process. The lysine content of the white ootheca (9.7 %) was considerably higher than that of the fully hardened ootheca (3.6 %) suggesting that lysine may be involved in the hardening process.

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Received November 16th, 1962

Biochim. Biophys. Acta, 71 (1963) 738–740

SC 2238

The use of specific amino acid decarboxylases for the identification of C-terminal groups

Although methods for N-terminal grouping have been adequately developed, there is no completely satisfactory method, as yet, for the characterisation of C-terminal groups. A widely used method, in which the C-terminal amino acid is released into the suspension medium by carboxypeptidase, provides results which may be difficult

to interpret. For example, if amino acids A and B are released into the suspension medium, this could mean either that A and B are both terminal groups; or that the two amino acids are the first and second residues in a protein chain. A new method of C-terminal grouping, using specific amino acid decarboxylases, has been developed and is particularly applicable to the study of the surface proteins of bacteria.

Cells of *Escherichia coli* (strain 101001), grown at 30° for 3 days in Davis minimal medium to which had been added 0.5 % (w/v) of the amino acid X, were harvested by centrifugation and acetone-dried. The X-decarboxylase was obtained, when required, as a cell-free extract by suspending the acetone-dried cells at 37° in buffer solution (pH 8.3) for 2 h.

C-terminal groups due to the amino acid X were detected by suspending the test organisms in the cell-free extract of X-decarboxylase at pH 5.0 for 16 h and observing the reduction of the electrophoretic mobility of the cells; this is a measure of the number of carboxyl groups removed.

Matt cells of a type 6 strain of *Streptococcus pyogenes* (N.C.T.C. 8302), referred to subsequently as type 6 M, from a 24-h culture were treated with hyaluronidase[1] and suspended in the decarboxylase extract. The suspension was adjusted to pH 5.0 and incubated at 37° for 16 h. The electrophoretic mobility of the decarboxylase-treated cells, measured by the method of GITTENS AND JAMES[2] at pH 7.5, was then compared with that of untreated cells. Cells treated with alanine decarboxylase or with glutamic acid decarboxylase had lower negative mobility values than the control cells, since the free carboxyl groups of these amino acids had been removed. Decarboxylases against the other 14 amino acids which we have identified in the surface protein of this strain did not reduce the mobility of the cells.

Cells of type 6 M from a 24-h culture were treated with hyaluronidase and then with fluorodinitrobenzene[3]; this blocked all amino groups and permitted study at low pH values. The resultant suspension was divided into three portions. The first sample was treated with alanine decarboxylase, the second with both alanine and glutamic acid decarboxylases and the third was the untreated control. The mobility values of the cells were determined at a number of pH values between 1.7 and 11.0,

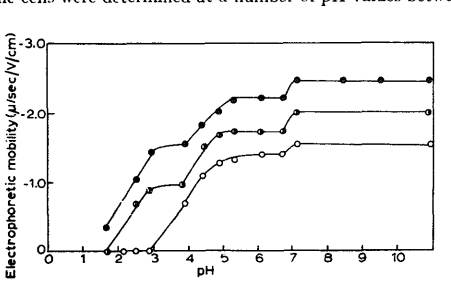

Fig. 1. pH-mobility curves of FDNB-treated cells of *Strep. pyogenes*, in buffer solution ($I = 0.02$), before and after treatment with alanine and glutamic acid decarboxylases. ●—●, FDNB-treated cells; ◑—◑, FDNB-treated cells after treatment with alanine decarboxylase; ○—○, FDNB-treated cells after successive treatments with alanine and glutamic acid decarboxylases.

$I = 0.02$ (Fig. 1). The control cells possessed groups with pK values of 2.1 and 4.1. Treatment of the cells with alanine decarboxylase reduced the number of groups of pK 2.1 by approx. 50 %. The surface of cells treated with both alanine and glutamic acid decarboxylase contained no groups of pK 2.1 but still possessed groups of pK 4.1—undoubtedly the γ-carboxyl groups of glutamic acid. The C-terminal groups of the surface of this organism were, therefore, the α-carboxyl group of alanine (pK 2.2) and the α- and γ-carboxyl groups of glutamic acid (pK 2.1 and 4.1 respectively). Table I shows the approximate densities of the three types of carboxyl groups calculated from the mobility values using the Gouy equation.

For this method to be successful, it is essential that the decarboxylase should be specific and should show no cross reactions. Since decarboxylation of the electrophoretically detectable carboxyl groups on the cells from 50 ml of culture will yield only 10^{-8} ml of carbon dioxide, it is not necessary for the enzyme extract to have

TABLE I

FREE CARBOXYL GROUPS ON THE SURFACE OF *Streptococcus pyogenes*

Type of carboxyl group	*Charge density (esu/cm²)*	*Charge ratio*
Alanine	650	1
α-Glutamic acid	660	1
γ-Glutamic acid	2090	3
Total	3400	

a high activity. Though the standard methods of preparing amino acid decarboxylases give preparations of very high activity, the complexity of the growth medium used is such that other decarboxylases will be present. Their presence will not be detectable by the normal assay method but they will be sufficiently active to invalidate results obtained using the electrophoretic method. The medium used in this work yields a preparation of the required enzyme whose activity is barely detectable by conventional methods, but which is specific and free from "cross-reactions".

Summarising, a method for the detection of the C-terminal groups at the surface of bacteria has been developed using bacterial amino acid decarboxylases in conjunction with the electrophoretic technique. The method is of limited applicability but, in our hands, gives unambiguous results.

One of us (M. J. H.) wishes to acknowledge the financial assistance of the Medical Research Council. We would also like to thank Dr. M. T. PARKER for the provision of laboratory facilities.

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Received November 21st, 1962