SC 11040

Localization of the "arginine dihydrolase system" in *Streptococcus faecium*

In a study of amino acid uptake by *Streptococcus faecium*[1] it was found that of the amino acids tested only arginine was taken up by this organism in the absence of glucose. However, although arginine disappeared from the medium none was found in boiling-water extracts of the cells, even after the shortest interval tested: instead, citrulline and ornithine were present. It was therefore of interest to determine whether or not the "arginine dihydrolase system"[2] of this organism was directly involved in the uptake of arginine. If this were so, then the components of the "dihydrolase system", arginine deiminase (L-arginine iminohydrolase, EC 3.5.3.6) and ornithine carbamoyltransferase (carbamoylphosphate:L-ornithine carbamoyltransferase, EC 2.1.3.3) might be expected to be present, and possibly localized, in its plasma membrane.

S. faecium was grown, harvested, and converted to protoplasts[3] by treatment with muramidase (EC 3.2.1.17; formerly known as lysozyme) in 0.5 M sucrose buffered with 75 mM potassium phosphate at pH 6.8. The resultant protoplasts were centrifuged, and a part of them resuspended in fresh, hypertonic sucrose while a second part was plasmolyzed in 1 mM potassium phosphate at pH 6.8. From a portion of the plasmolyzed protoplasts, cytoplasmic and membrane fractions were prepared by differential centrifugation[4]. In addition, a part of the cell harvest was directly lysed by muramidase treatment in 75 mM potassium phosphate buffer (pH 6.8).

Intact cells, lysed cells, protoplasts, lysed protoplasts, cytoplasmic and membrane fractions, and a wall fraction (the supernatant obtained from centrifugation of the lysozyme–cell mixture in hypertonic medium) were incubated either in 9 mM L-arginine or 11 mM L-citrulline at pH 6.8; enough sodium arsenate was added to both mixtures to make them 10 mM (see ref. 5). Zero-time and 30-min samples were withdrawn and brought to 5% trichloroacetic acid with conc. trichloroacetic acid. After holding overnight at 4°, the samples were cleared by centrifugation.

The supernatants were spotted on Reeve Angel SA-2 ion-exchange paper in the Na^+ form and the paper was developed with 0.2 M sodium acetate (pH 5.2)[6]. After development the chromatograms were treated with the ninhydrin reagent of HEILMANN *et al.*[7]. The resultant colored areas were eluted with an 80% acetic acid–1% Tween-80 mixture containing 25 mg/ml of cadmium acetate, and the absorbancies of the eluants at 500 mμ compared with those of appropriate standards. Duplicate determinations were also made of arginine and citrulline, respectively, by a modified Sakaguchi reaction[8] and a modified Archibald reaction[5]. (However, severe interference was experienced using the latter method with samples in 0.5 M sucrose.) Protein was estimated by biuret determinations on the lysed-cell samples.

The distribution of enzyme activity among the cells and cell fractions is shown in Table I.

Both enzymes are concentrated in the cytoplasm, and the plasma membrane is essentially devoid of their activity.

The deiminase activity observed in the wall fraction probably represents material from cells damaged during protoplast preparation rather than its occurrence in the wall, since the specific activity in this fraction is very similar to that observed in

TABLE I

DISTRIBUTION OF ARGININE DEIMINASE AND ORNITHINE CARBAMOYLTRANSFERASE ACTIVITIES IN CELLS AND CELL FRACTIONS OF *Streptococcus faecium*

Fraction	Arginine deiminase		Ornithine carbamoyltransferase		
	Specific activity* × 10^3	Per cent of intact cell activity shown by fraction	Specific activity* × 10^3	Per cent of lysed-cell activity shown by fraction	Per cent of lysed-cell protein in fraction
Intact cells	74 (56–89)**	—	1 (0–5)	13 (0–51)	
Lysed cells	66 (47–82)	89 (81–100)	8 (7–11)	—	—
Wall fraction	52 (18–76)	8 (4–14)	16 (16–17)	22 (17–26)	10 (10–10)
Protoplasts	81 (71–95)	90 (72–100)	2 (0–3)	15 (0–33)	82 (79–84)
Lysed protoplasts	83 (75–88)	96 (80–106)	13 (9–17)	140 (100–175)	85 (74–95)
Cytoplasmic fraction	108 (106–112)	93 (83–106)	22 (14–29)	150 (118–180)	63 (55–70)
Membrane fraction	9 (0–19)	3 (0–5)	0 (0–0)	0 (0–0)	20 (17–22)

* Determination of μmoles of substrate disappearing per min per mg protein at 25° averaged with the determination of the μmoles of amino acid products appearing per min per mg protein at 25°.

** The mean, followed by the observed range, of 4 determinations.

intact and in lysed cells. In either case, the amount of deiminase activity found in this fraction is less than 10%, on the average, of that found in the whole cell.

In the case of the carbamoyltransferase activity found in the wall fraction, its specific activity approximates more nearly that of the lysed cells and lysed protoplasts, rather than intact cells or protoplasts which do not readily take up citrulline, suggesting that its presence in the wall fraction is also a result of damage to cells during their conversion to protoplasts, particularly since the intact cells exhibit the same per cent of lysed-cell activity as the protoplasts, rather than a per cent activity which is the sum of the activities of the protoplast and the wall fractions.

The considerably higher activity of the carbamoyltransferase in the protoplast lysate and in the cytoplasmic fraction than in the cell lysate, although consistently observed, is without explanation at present.

The cytoplasmic fraction was subjected to centrifugation at 105 000 × *g* for 2 h and the supernatant and sediment examined for both enzyme activities. None of the deiminase activity was sedimented, while about 30% of the carbamoyltransferase activity was in the pellet. At least part of the ornithine carbamoyltransferase, then, is bound to cell particulates.

The absence of the two enzymes from the membrane fraction makes it unlikely that either of them is directly involved in the uptake of arginine. Their activity, in fact, provides a means of demonstrating that arginine has penetrated the plasma membrane and reached the cell's interior, distinguishing arginine penetration in the cell from its absorption to the cell surface[3].

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Interference by reactions of kynurenine metabolism in the estimation of tryptophan pyrrolase in rat-liver homogenate

In an investigation of aspects of the mechanism of tryptophan pyrrolase induction using a continuous-perfusion technique[1], the results of which are to be published elsewhere, a tryptophan pyrrolase assay was established using minor modifications of the method outlined by KNOX[2]. The significant modification was the use of a Dounce, hand-operated homogenizer[3] instead of a Waring blendor for dispersion of the tissue. Under these conditions, in contrast to those of previous workers[4,5], it was found that the kynurenine synthesized during incubation of the homogenate with tryptophan was being further metabolized. Therefore, the kynurenine content of the incubation mixture at the end of the incubation period was not a true measure of the tryptophan pyrrolase activity.

The incubation mixture (total volume 3.0 ml and final pH 7.0) contained 9 μmoles L-tryptophan, 150 μmoles sodium phosphate buffer and 125 mg rat liver as an homogenate (12.5%, w/v, in 0.14 M KCl). Incubation was for 2 h at 37°.

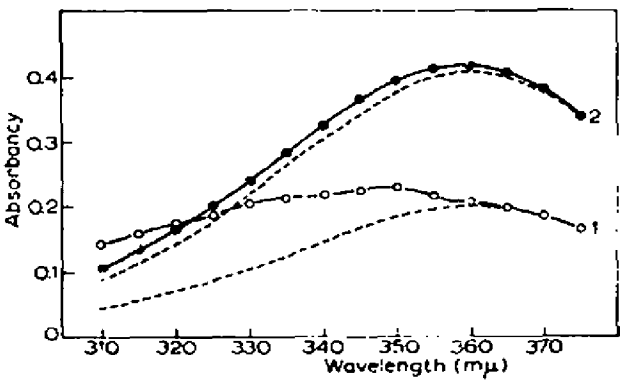

Fig. 1. Absorption spectra of the reaction products of the tryptophan pyrrolase assay using an homogenate freshly prepared with a Dounce homogenizer and using part of the same homogenate after it had been frozen in a dry ice–acetone mixture and immediately thawed. Conditions of assay were as outlined in the text. Values represent the means of three determinations. ○—○, freshly prepared homogenate; ●—●, frozen and thawed homogenate; — — —, authentic L-kynurenine.