chloride are replaced by choline and sulphate there is no significant change in the negative potential, indicating that the negative polarity is not due to movements of chloride. It is surprising that replacement of potassium does not affect bioelectric measurements; however, there may be enough potassium leaking from the muscular portion of the stomach to ensure maintenance of a finite, local concentration; the media, originally potassium-free, shows an assayed potassium-level of 0·4 mmolar after 1 h.

These results show that the potential difference measured across the isolated gastric mucosa is dependent on sodium and not chloride and that the potentials observed are comparable to those reported by Wright in the fœtal rabbit[6]. While a chloride pump probably operates *in vivo* along with a sodium pump, one should note that Wright[6] also gave evidence that hydrochloric acid secretion is small and restricted to oxyntic cells. The nature of the other potential, namely, of −2 mV, must still be clarified. This potential, while not dependent on sodium or chloride ions from the surrounding media, nevertheless is affected by tissue anoxia. Preliminary investigations by double label sodium tracers have shown a resultant net flux of 6·0 μmoles Na^+/h from mucosa to serosa, a value which is abolished by nitrogen and which agrees in magnitude with the ionic equivalent of the short circuit current[9].

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BIOCHEMISTRY

Enzymatic Synthesis of *S*-Sulphocysteine from Thiosulphate and Serine

Sulphate assimilation in *Aspergillus nidulans* has been shown to involve a step in which thiosulphate reacts with a 3-carbon compound, probably serine, to form *S*-sulphocysteine as the immediate precursor of cysteine[1–5]. However, the enzymatic mechanism involved in this assimilatory step has not yet been elucidated. This communication shows that an enzyme preparation obtained from *A. nidulans* catalyses the condensation of thiosulphate with serine to form *S*-sulphocysteine if adenosine triphosphate (ATP) and pyridoxal phosphate are added as cofactors.

The enzyme preparation used was extracted from acetone-dried mycelia of *A. nidulans* by grinding with quartz sand, freed from particulate matter by centrifugation at 105,000*g* for 90 min, precipitated with ammonium sulphate at 80 per cent saturation, and dialysed against 1 mM *tris*-hydrochloric acid buffer, *p*H 8·0. The condensation reaction was demonstrated by determining the incorporation of labelled thiosulphate into *S*-sulphocysteine after separating the latter by column chromatography. The complete reaction mixture contained, in a total volume of 12·5 ml., the enzyme preparation (90 mg of protein), 50 μmoles each of L-serine and thiosulphate labelled with sulphur-35 (inner sulphur atom labelled, 0·1–0·3 mc.), 25 μmoles of pyridoxal phosphate, 30 μmoles each of ATP and magnesium chloride, and 125 μmoles of *tris*-hydrochloric acid buffer, *p*H 8·0. The incubation was carried out aerobically at 30° for 90 min.

At the end of incubation, 12·5 μmoles of *N*-ethylmaleimide in 0·24 ml. of ethanol was added to the reaction system to stop the reaction and to protect *S*-sulphocysteine from decomposition by thiols[4,5]. The mixture was then mixed with 10 mg of authentic sodium *S*-sulpho-L-cysteine as carrier and deproteinized by gel filtration through a 'Sephadex *G*-50' column. The deproteinized solution retaining almost all of the radioactivity was applied to a Dowex-1 formate column and eluted with 1 M pyridine formate buffer[6] at *p*H 5·4 as previously described[5]. *S*-sulphocysteine could thereby be obtained in a fraction which was almost free from the other sulphur compounds and ninhydrin-positive substances.

The sulphocysteine fraction thus obtained contained 7·4 per cent of the original radioactivity, indicating that 3·7 μmoles of thiosulphate had been incorporated into the sulphocysteine formed. The sulphocysteine was then decomposed by performic acid oxidation into inorganic sulphate and cysteic acid according to the previously described method[5]. It was thus found that the sulphate fraction, which must have been derived from the inner sulphur atom of the added thiosulphate, contained about 80 per cent of the radioactivity of the isolated sulphocysteine. The cysteic acid fraction, on the other hand, could account for only 2 per cent of the total radioactivity. Since the thiosulphate added had been labelled at the inner sulphur atom, these data indicate that the thiosulphate as such had been incorporated into *S*-sulphocysteine without splitting of the disulphide bond.

Table 1. Incorporation of Labelled Thiosulphate into *S*-Sulphocysteine under Different Conditions

Enzyme source	Reaction system	Thiosulphate incorporated into *S*-sulphocysteine (μmole)
Wild-type strain	Complete	3·7
,, ,, ,,	,, (heated enzyme)*	0
,, ,, ,,	,, (anaerobic)	0·4
,, ,, ,,	Minus serine	0
,, ,, ,,	Minus ATP and $MgCl_2$	0·6
,, ,, ,,	Minus pyridoxal phosphate	0·5
Strain 721	Complete	0·8
Strain 793	Complete	6·8

* Heated at 80° for 5 min.

In Table 1 are recorded the results of thiosulphate incorporation experiments conducted under various conditions. It will be seen that the enzyme system is heat-labile and does not incorporate thiosulphate into *S*-sulphocysteine in the absence of serine. Little incorporation could be observed when the reaction was carried out under anaerobic conditions, but the reasons for this inhibition by anaerobiosis are not yet clear. The most interesting feature of the condensation reaction may, however, be the fact that the omission of either ATP (plus magnesium chloride) or pyridoxal phosphate from the system results in practically no synthesis of *S*-sulphocysteine. The requirement of ATP strongly suggests that thiosulphate must be activated, probably in a similar way as in the case of biological sulphate activation[7], prior to its condensation with serine. It is also likely that pyridoxal phosphate is required as a cofactor in the reaction of the activated thiosulphate with serine.

Table 1 further shows that the thiosulphate-incorporating activity is very low in the enzyme preparation obtained from strain 721, a mutant of *A. nidulans* requiring *S*-sulphocysteine, cysteine or methionine for growth[2,3]. On the other hand, the preparation extracted from strain 793, in which the conversion or *S*-sulphocysteine to cysteine is genetically blocked[2–5], showed a high activity of the condensing enzyme system. These findings indicate clearly that the incorporation of thiosulphate into *S*-sulphocysteine is an essential step in sulphate assimilation in *A. nidulans*.

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