III has a mol.wt. of less than 7000 or alternatively that its structure is such that it is strongly bound to and retarded on Sephadex columns. About 90% of the enzyme activity placed on the column was recovered.

The material in fraction I when rechromatographed on Sephadex G-100 under the same conditions separated again into three peaks of activity in which the last peak predominated (Fig. 1).

Peak III has properties associated with enzyme proteins in general. The optimum pH for enzyme activity is 8.2 (Fig. 2*a*). The enzyme is relatively heat-stable, losing activity at temperatures in excess of 45°C (Fig. 2*b*).

Discussion

The results of the present studies would suggest that asparagine synthetase in the asparaginase resistant lymphoma 6C3HED consists of one form of mol.wt. 70000. This material, under appropriate conditions, appears to undergo fragmentation, yielding in part a molecule that has a low molecular weight but that is enzymatically active. This small fragment might represent a subunit form of the enzyme that is produced as a result of disulphide bond cleavage or alternatively it might result from a proteolytic cleavage of that form of mol.wt. 70000. Whatever the origin, this enzymically active low-molecular-weight form of enzyme is of interest. It might be noted that asparagine synthetase isolated in a highly purified form from RADAI cells (recovery 9%) was shown to have a mol.wt. of just over 100000 (Horowitz & Meister, 1972).

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Evidence from ^{31}P Nuclear Magnetic Resonance that Polyphosphate Synthesis is a Slip Reaction in *Paracoccus denitrificans*

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Two outstanding problems in prokaryotic bioenergetics (for example, see Stouthamer, 1977; Neijssel & Tempest, 1976) are the following: (1) How do bacteria effectively

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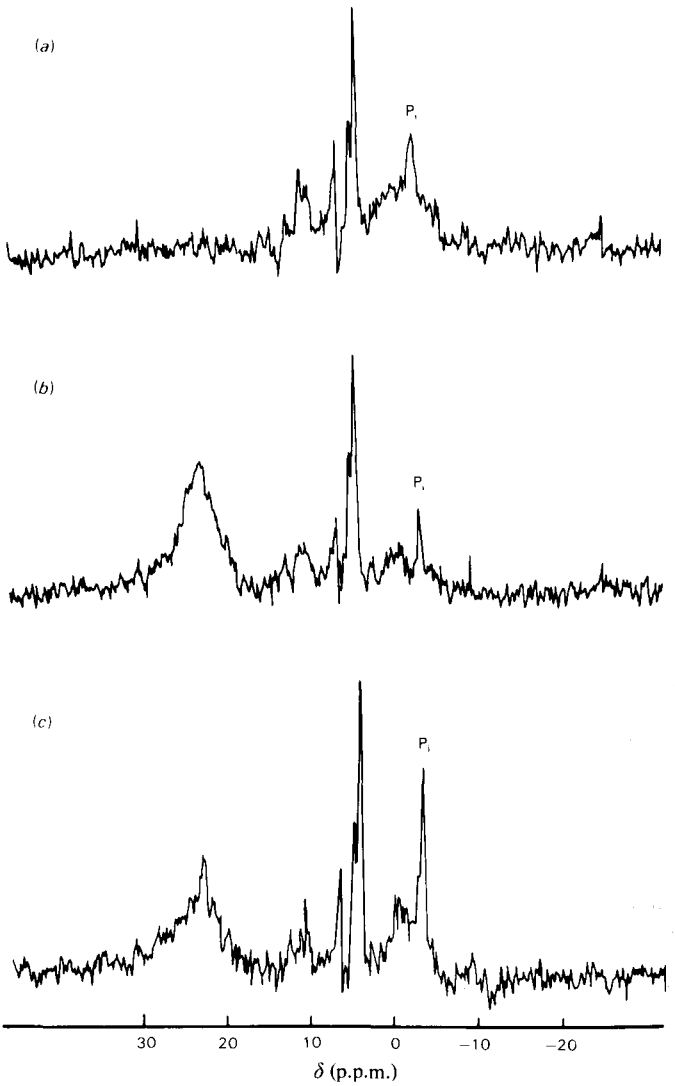

Fig. 1. ^{31}P *n.m.r. spectra of P. denitrificans cells*

P. denitrificans cells, grown anaerobically with succinate as carbon source and nitrate as electron acceptor (Burnell *et al.*, 1975), were suspended after washing in 150mM-NaCl, 10mM-Tris/HCl, pH 8.0, at 75mg dry weight per ml. The n.m.r. spectra were collected at room temperature using the spectrometer mentioned in Hoult *et al.* (1974). (*a*) Resting cells, 500 scans accumulated over 15min. (*b*) After addition of 50mM-sodium succinate and 100mM-KNO_3. The collection of the n.m.r. spectrum (500 scans over 15min was started approx. 1h after addition of succinate and nitrate. (*c*) After addition of 10μM-carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone. The collection of the n.m.r. spectrum (500 scans over 15min after addition of the uncoupler. The same suspension of cells was used for the three experiments. There is an apparent resonance at approx. 6 p.p.m. which is an instrumental artefact.

dissociate respiration from growth, or more specifically why is there an immediate increase in the rate of consumption of O_2 (or other electron acceptor) when oxidizable substrate is added to a washed suspension of bacteria, and what is the fate of any energy (ATP) thus generated? (2) What is the role of polyphosphate? The value of ^{31}P n.m.r for studying metabolism in tissues (e.g. Hoult *et al.*, 1974) and micro-organisms (e.g. Salhany *et al.*, 1975; Ugurbil *et al.*, 1978) is now well documented, and the present report is concerned with some experiments that we have initiated using ^{31}Pn.m.r. to examine these questions in *Paracoccus denitrificans*.

The ^{31}P n.m.r. spectrum of a suspension of washed resting cells of *P. denitrificans* is shown in Fig. 1(*a*). Few of the resonances have been firmly assigned, but for the present purposes the following conclusions are useful. (*a*) The P_i peak is at a chemical shift that indicates an intracellular pH of 7.2 (cf. Scholes & Mitchell, 1970). (*b*) The ATP concentration is very low as judged by the absence of a resonance in the region of 20p.p.m., which is the chemical shift for the β-phosphate of ATP at this pH. (*c*) There are very prominent peaks from a number of unidentified metabolites.

When succinate and nitrate were added to the suspension of cells, the spectrum shown in Fig. 1(*b*) was obtained. Parenthetically we would point out that the use of nitrate as added terminal electron acceptor should result in the cells respiring homogeneously and continuously in concentrated suspension; obtaining similar results with O_2 is difficult. Fig. 1(*b*) shows that a very large and broad resonance develops, which can be assigned to inorganic polyphosphate (cf. Salhany *et al.*, 1975). At the same time the resonance from P_i shifts, indicating that the intracellular pH has risen to at least pH7.6, but the intensity of the P_i resonance is only slightly diminished. Comparison of Fig. 1(*a*) with Fig. 1(*b*) shows that the broad resonance(s) (presumably due to macromolecular phosphorus) under the P_i resonance in Fig. 1(*a*) has diminished upon initiating respiration. The relative areas of the original P_i resonance and the polyphosphate resonance indicate that the source of phosphorus for polyphosphate synthesis cannot be the original cellular pool of P_i; presumably it is phosphate derived from breakdown of macromolecules including, probably, those that contain phosphodiester bonds. As polyphosphate is synthesized by transfer of the terminal phosphate group of ATP to polyphosphate (Harold, 1966), we conclude that at least part of the energy released by succinate oxidation is arst conserved as ATP, and thus polyphosphate production may account for a significant part of the 'slip reaction' (Neijssel & Tempest, 1976) in *P. denitrificans*.

Fig. 1(*c*) shows a ^{31}P n.m.r. spectrum obtained on adding an uncoupler of oxidative phosphorylation to the cell suspension which had previously given the spectrum shown in Fig. 1(*b*). Uncoupling results in a decrease in the intensity of the polyphosphate resonance and a considerable increase in the intensity of the P_i resonance, thus indicating breakdown of polyphosphate. Even when considerable polyphosphate remains, the P_i resonance is much larger than before respiration was initiated (compare Fig. 1*a* with 1*c*), which again indicates that the original source of P_i for polyphosphate formation was probably from macromolecules. An important question is whether ATP concentrations are maintained during the breakdown of polyphosphate (Harold, 1966). It is not possible to decide this point from the data presented here because the characteristically distinct resonance from the β-phosphate of ATP overlaps with the signal from polyphosphate, and the spectra are as yet incompletely assigned.

From these exploratory ^{31}P n.m.r. experiments the following conclusions may be drawn. (*a*) One of the slip reactions in *P. denitrificans* is the consumption of ATP to form polyphosphate. The source of P_i for this polyphosphate synthesis is not the initial P_i pool in the cell. (*b*) The pH inside the cells rises upon initiating resporation; the pH of the suspending medium reached pH8.5. In general it is not known how stringently respiring bacterial cells control their pH (cf. Riebeling *et al.*, 1975). (*c*) The polyphosphate breaks down in the presence of an uncoupling agent, but it is not known if this breakdown maintains the ATP concentration in the cell. This result contrasts with the finding in *Neurospora crassa* (Harold, 1966) that polyphosphate does not break down

if energy generation is limited or blocked, and suggests that polyphosphate may be an energy store as well as a phosphate store in *P. denitrificans*.

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The Relationship between Proton Translocation and Cell Yields in the Facultative Methylotroph *Pseudomonas AMI* and a Mutant Lacking Cytochrome *c*

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In a comprehensive survey of a range of different bacteria Jones *et al.* (1977) have shown that a clear correlation exists between electron-transport components, proton translocation and cell yields. Their results demonstrated that the efficiency of aerobic bacterial growth on a particular carbon source was determined predominantly by the presence or absence of a high-potential membrane-bound cytochrome *c*. The present communication concerns the relationship between cell yields and the stoicheiometry of proton translocation in *Pseudomonas* AMI and in a mutant lacking cytochrome *c* (mutant PCT76). *Pseudomonas* AMI is a facultative methylotroph and the absence of cytochrome *c* in mutant PCT76 prevents growth on C_1 compounds while leaving growth on multicarbon compounds such as succinate unaffected (Anthony, 1975; Widdowson & Anthony, 1975). Furthermore, the stoicheiometry of proton translocation was the same in wild-type and mutant bacteria (in batch-grown cells) and it has therefore been suggested that cytochrome *c* may never be involved in the oxidation of succinate of NADH in *Pseudomonas* AMI (O'Keeffe & Anthony, 1978). To test this possibility further, wild-type and mutant bacteria were grown in continuous culture, and cell yields and the stoicheiometry of proton translocation measured.

Bacteria were grown aerobically at 30°C in the medium described by Maclennan *et al.* (1971) and $\rightarrow H^+/O$ ratios measured as previously described (O'Keeffe & Anthony 1978). When grown in continuous culture the pH was controlled at pH 7.0 and the air supply was regulated to achieve 60–80% dissolved oxygen tension. The oxygen concentration was measured in inflowing and effluent gas with an Oxygen Analyser (type OA272; Taylor Servomex Ltd.). The concentrations of methanol and succinate in inflowing and effluent growth media were measured by gas chromatography and the dry weight of the culture was measured directly.

The results in Table 1 show that the stoicheiometry of proton translocation (the $\rightarrow H^+/O$ ratio) during endogenous respiration was about 4 in batch-grown bacteria, in NH_4^+-limited continuous cultures and in bacteria taken from a methanol-limited continuous culture during transition from a lower to a higher growth rate; this confirms