Catabolism of Adenosine 5'-Monophosphate by Extracts of the Marine Bacterium *Beneckea natriegens*

By DONALD F. NIVEN,* PAULINE A. COLLINS
AND CHRISTOPHER J. KNOWLES

Biological Laboratories, University of Kent,
Canterbury CT2 7NJ

(Received 25 November 1976)

SUMMARY

AMP is catabolized by cell-free extracts of *Beneckea natriegens* to inosine via adenosine by AMP nucleotidase and adenosine deaminase. In the presence of ATP, the AMP nucleotidase is inhibited and an AMP deaminase is activated, resulting in formation of IMP. When low concentrations of ATP are used, the IMP is converted, simultaneously with ATP consumption, to inosine by IMP nucleotidase, which is presumably ATP-sensitive. Since 5'-nucleotidases from various organisms are known to catabolize several ribonucleoside monophosphates, the AMP and IMP nucleotidase activities of *B. natriegens* may be due to the same enzyme. CTP, GTP and UTP inhibit AMP nucleotidase from *B. natriegens* without stimulating AMP deaminase, thus severely decreasing the rate of AMP breakdown.

INTRODUCTION

Whereas other aspects of adenine nucleotide metabolism by living organisms have been extensively investigated, there have been relatively few studies of AMP catabolism, particularly in bacteria (Drummond & Yamamoto, 1971). Possible pathways of AMP catabolism are shown in Fig. 1.

AMP nucleotidase has been found in several bacteria (Kohn & Reis, 1963; Heppel, 1971; Felicioli *et al.*, 1973) and, where studied, it has been shown to be located in the periplasm (Heppel, 1971). In *Escherichia coli* it is involved in the uptake of exogenous AMP (Yagil & Beacham, 1975). Mutants of *E. coli* deficient in AMP nucleotidase are unable to take up AMP but are otherwise able to grow normally: therefore, this enzyme probably does not have an essential role in the catabolism of intracellular AMP (Yagil & Beacham, 1975).

In *Azotobacter vinelandii*, AMP is degraded to adenine by AMP nucleosidase, and then to hypoxanthine by adenine deaminase (Hurwitz, Heppel & Horecker, 1957; Schramm & Leung, 1973; Schramm & Lazorik, 1975). *Escherichia coli* contains AMP nucleosidase but not adenine deaminase (Koch & Vallee, 1959; Schramm & Lazorik, 1975). In contrast, AMP nucleosidase has not so far been found in any eukaryotes (Schramm & Leung, 1973). In mammalian tissues AMP is catabolized to inosine by AMP deaminase and IMP nucleotidase and/or by AMP nucleotidase and adenosine deaminase (Burger & Lowenstein, 1967; Woods, Eggleston & Krebs, 1970; Drummond & Yamamoto, 1971). AMP deaminase has been extensively purified from several eukaryotes and its properties have been studied (cf. Smith & Kizer, 1969; Boosman & Chilson, 1976; Makarewicz & Stankiewicz, 1976).

* Present address: Department of Microbiology, Macdonald Campus of McGill University, Macdonald College P.O., Province of Quebec HoA 1Co, Canada.

Fig. 1. Possible pathways of AMP catabolism. AMP, adenosine 5'-monophosphate; IMP, inosine 5'-monophosphate; R, D(-)-ribose; RP, ribose 5-phosphate; P_i , inorganic phosphate.

Enzymes: 1, AMP deaminase (EC 3.5.4.6); 2, IMP nucleotidase (EC 3.1.3.5); 3, AMP nucleotidase (EC 3.1.3.5); 4, adenosine deaminase (EC 3.5.4.4); 5, AMP nucleosidase (EC 3.2.2.4); 6, IMP nucleosidase (EC 3.2.2.12); 7, inosine phosphorylase (EC 2.4.2.1); 8, inosine nucleosidase (EC 3.2.2.2); 9, adenosine phosphorylase (EC 2.4.2.1); 10, adenosine nucleosidase (EC 3.2.2.7); 11, adenine deaminase (EC 3.5.4.2).

Though a non-specific adenine nucleotide deaminase (EC 3.5.4.17) has been found in *Desulfovibrio desulfuricans* (Yates, 1969), there have been no reports of the presence of AMP deaminase in bacteria.

Overall metabolic activity of growing organisms is controlled by the action of ATP, ADP and AMP as allosteric effectors of a wide range of enzymes (Atkinson, 1968; Knowles, 1977). The size of the adenine nucleotide pool and its proportional make-up, expressed as an energy charge of 0.8 to 0.9, are strictly controlled during growth of bacteria (Chapman, Fall & Atkinson, 1971; Niven, Collins & Knowles, 1977; Knowles, 1977).

Does AMP catabolism have a role in maintaining the adenine nucleotide pool during growth? If not, how is its breakdown regulated so that depletion of the adenylates does not occur? Liver AMP deaminase and AMP nucleosidase from *A. vinelandii* are activated by a decrease in energy charge (see Atkinson, 1968) and inhibited by inorganic phosphate (Chapman & Atkinson, 1973; Schramm & Leung, 1973). It has been postulated that these enzymes have a similar role of temporarily protecting metabolic activity of the tissue or organism by causing a transient decrease in the total adenine nucleotide pool size and thus maintaining energy charge under conditions of environmental stress (Chapman & Atkinson, 1973; Schramm & Leung, 1973).

Recent experiments on the adenine nucleotide content of growing *Beneckea natriegens* have shown that, on starvation, the energy charge is maintained at the growth value of about 0.9 for several hours and there is little decrease in total nucleotide content (Niven *et al.*, 1977). We have therefore examined the pathways of AMP catabolism by *B. natriegens* in order to determine how the adenine nucleotide content is regulated. This paper reports that AMP is broken down to inosine via adenosine by AMP nucleotidase and adenosine deaminase but, in the presence of ATP, it is broken down to IMP by AMP deaminase. AMP deaminase has not previously been observed in a bacterium.

METHODS

Organism and growth conditions. *Beneckea natriegens* strain III was grown in a defined medium (Niven *et al.*, 1977) containing 10 mM-sodium succinate. Overnight starter culture (8 ml) was inoculated into 400 ml fresh medium in 2 l baffled flasks and grown at 35 °C on a rotary shaker (200 rev. min⁻¹). Bacteria were harvested in the mid-exponential phase of growth ($E_{680\text{ nm}}$ approx. 0.7 in 10 mm cuvettes in a Pye Unicam SP500 spectrophotometer).

Preparation of extracts. Bacteria from four 400 ml cultures were centrifuged at 23000 *g* for 10 min, washed with 50 ml 100 mM-triethanolamine/HCl containing 300 mM-NaCl, 30 mM-KCl, 2.22 mM-MgSO₄, 0.1 mM-Na₂EDTA, 0.1 mM-dithiothreitol, and 3 μ M-phenylmethylsulphonyl fluoride (pH 8.0), and resuspended in 3 ml fresh buffer. Bacteria were disrupted by sonication (MSE sonicator, 150 W, 4 $\times$ 15 s 0 °C) and, after centrifuging at 12000 *g* for 10 min at 4 °C, the cell-free extract was either dialysed overnight against 500 ml fresh buffer at 4 °C or fractionated into particulate and supernatant fractions as described previously (Weston & Knowles, 1973, 1974). The particulate fraction was resuspended in 1 ml fresh buffer and the particulate and supernatant fractions were dialysed as above. Protein was assayed by the modified biuret method (Gornall, Bardawill & David, 1949).

Determination of enzyme activities. Adenine, adenosine and AMP deaminases were assayed as described by Schramm & Lazorik (1975) except that the reaction mixture contained (in 1.5 ml) either dialysed cell-free extract (10 μ l), dialysed supernatant fraction (10 μ l) or dialysed particulate fraction (2.5 μ l) in 90 mM-triethanolamine/HCl containing 270 mM-NaCl, 27 mM-KCl, 2 mM-MgSO₄, 90 μ M-Na₂EDTA, 90 μ M-dithiothreitol, and 2.7 μ M-phenylmethylsulphonyl fluoride (pH 8.0), and either 20 μ M adenine, adenosine or AMP. AMP nucleosidase, adenosine nucleosidase and adenosine phosphorylase were assayed as described by Schramm & Lazorik (1975) except that 2 μ l dialysed cell-free extract was used in the above buffer system.

Degradation of radioactive substrates. Dialysed cell-free extract, supernatant fraction or particulate fraction (180 μ l) was incubated for 30 min at 30 °C and the reaction was then started by adding 50 μ l [U-¹⁴C]AMP, [U-¹⁴C]ATP or [U-¹⁴C]inosine (12.5 mM, 1.6 mCi mmol⁻¹, pH 8.0) or 50 μ l [³²P]AMP (12.5 mM, about 3 mCi mmol⁻¹, pH 8.0). Other nucleotides (20 μ l, pH 8.0) or buffer were added at the same time as the radioactive source. Samples (20 μ l) were removed at intervals and added to 1 ml 67% (v/v) ethanol at room temperature. Ethanolic samples were immediately transferred to a water bath at 80 °C for 3 min and then removed to ice. They were rapidly freeze-dried (< 4 h) and stored at -22 °C until chromatographed.

Chromatography and radioactivity counting. Freeze-dried samples from experiments with ¹⁴C-labelled substrate were reconstituted with 0.25 ml water and 5 μ l samples were spotted on to thin-layer cellulose plates (no. 6065 with fluorescent indicator, Eastman Kodak). When [³²P]AMP was used as the substrate an additional step was introduced to remove released inorganic [³²P]phosphate, which interfered with the thin-layer chromatography. Freeze-dried samples were reconstituted with 1 ml 0.1 M-HCl and applied to a 0.5 $\times$ 0.5 cm column containing about 1 mg Norit A activated charcoal, which had been pre-treated to partially deactivate it by stirring with 15% (v/v) octan-2-ol in ethanol followed by washing in ethanol, then water, and finally drying at 100 °C (Smith, Kelly & Roy, 1974). After applying the labelled material to the column, it was washed with 5 ml 0.1 M-HCl and eluted with 2 ml ethanol/ammonia (sp.gr. 0.88)/water (45:5:50, by vol.). The eluate was

spotted on to thin-layer plates. Chromatography and liquid scintillation counting were performed as described by Schramm & Lazarik (1975).

Chemicals. ATP (disodium salt), CTP (type V), UTP (type I, sodium salt), ADP (grade I, sodium salt), AMP (type II, sodium salt), IMP (sodium salt), adenine, inosine, hypoxanthine, ribose 5-phosphate (disodium salt), octan-2-ol, 2,9-dimethyl-1,10-phenanthroline hydrochloride, dithiothreitol, glycine, phenylmethanesulphonyl fluoride, triethanolamine hydrochloride, Tris (Trizma base) and activated charcoal (Norit A) were obtained from Sigma. [U- ^{14}C]AMP, [U- ^{14}C]ATP and [U- ^{14}C]inosine were obtained from The Radiochemical Centre, Amersham. [^{32}P]AMP was a gift from Dr R. Martin (University of Cambridge). GTP (trisodium salt) was obtained from Boehringer, adenosine (A grade) from Calbiochem, and 2,5-diphenyloxazole and 1,4-di-2-(5-phenyloxazolyl)benzene (both scintillation grade) from Koch-Light. Whenever possible, all other reagents were of analytical grade; glass-distilled water was used throughout.

RESULTS

Spectrophotometric or colorimetric assays were made of several enzymes involved in AMP catabolism to determine which occur in *B. natriegens*. Only AMP deaminase [3.3, 1.6 and 4.1 nmol min⁻¹(mg protein)⁻¹ in the cell-free extract, particulate fraction and supernatant fraction, respectively] and adenosine deaminase [6.1, 4.3 and 8.1 nmol min⁻¹(mg protein)⁻¹, respectively] were found; the observed activities must be considered approximate due to the possibility of interfering cross-reactions. Adenine deaminase, AMP nucleosidase, and adenosine nucleosidase and phosphorylase activities were not detected.

Unfortunately, detection of these enzyme activities is insufficient to demonstrate the pathway of AMP catabolism. Furthermore, assays of this type cannot be used with crude extracts to determine whether AMP nucleotidase is present. In order to elucidate the AMP catabolic pathway(s), [^{14}C]AMP was added to extracts and the products were analysed.

When [^{14}C]AMP was catabolized by cell-free extracts in the absence of ATP there was a rapid build-up of inosine: no breakdown of inosine to hypoxanthine occurred and about 90 % of the applied ^{14}C was recovered as inosine (Fig. 2*a*). Adenosine formation (up to 35 % of the total ^{14}C) preceded the appearance of inosine, suggesting that the primary route of AMP catabolism is via AMP nucleotidase and adenosine deaminase. However, some inosine could also be formed by the action of AMP deaminase plus IMP nucleotidase, with IMP not being detected due to the former enzyme having a lower activity under these conditions.

AMP catabolism was also measured in the particulate and supernatant fractions obtained by centrifuging the cell-free extract at 150000 g (Fig. 3). AMP was broken down about eight times more rapidly by the particles than by the supernatant fraction (Fig. 3*a, d*). Furthermore, the particulate fraction converted 85 % of the AMP to adenosine before inosine was formed (Fig. 3*a*); thus AMP must be entirely catabolized by AMP nucleotidase and adenosine deaminase. In contrast, only about 40 % of the ^{14}C was found as adenosine in the supernatant fraction (Fig. 3*d*), presumably due to the greater concentration of adenosine deaminase relative to AMP nucleotidase in this fraction.

Mammalian AMP deaminases are activated by ATP, yet inactivated by increases in energy charge in the 0.6 to 0.9 region (Burger & Lowenstein, 1967; Smith & Kizer, 1969; Drummond & Yamamoto, 1971; Chapman & Atkinson, 1973). On the other hand, several AMP nucleotidases are inhibited by ATP (Burger & Lowenstein, 1967; Woods *et al.*, 1970; Drummond & Yamamoto, 1971).

Fig. 2. The catabolism of 2.5 mM-[U- 14 C]AMP by cell-free extracts of *Beneckea natriegens* in the absence of ATP (a), and in the presence of ATP at 5 mM (b), 10 mM (c) and 40 mM (d): $\circ$, AMP; $\bullet$, adenosine; $\blacktriangle$, inosine; $\triangle$, IMP. No results are shown for ribose 5-phosphate, hypoxanthine, adenine, and, where applicable, adenosine, IMP and inosine; for each of these compounds, counts were less than 6% of the total. Protein concentrations were (mg ml $^{-1}$): (a) 12; (b), (c) and (d) 24.

In preliminary experiments on the breakdown of 1 mM-[14 C]ATP by cell-free extracts of *B. natriegens*, the energy charge rapidly decreased from 1.0 to 0.3 and the ATP disappeared within a few minutes (not shown). Over 90% of the 14 C was recovered as inosine, after the intermediary formation of adenosine. However, despite this conversion of ATP to ADP and AMP by ATPase, adenylate kinase and other enzymes, the effect of ATP and/or high energy charge values on the catabolism of [14 C]AMP could be measured in the presence of 40 mM unlabelled ATP: during the period of the experiment only a small portion of the total ATP was converted to other adenylates and the energy charge and ATP content remained high.

In the presence of 40 mM-ATP, 2.5 mM-[U- 14 C]AMP was converted to IMP (> 90% recovery of 14 C) by cell-free extracts, suggesting the presence of an ATP-activated AMP deaminase (Fig. 2d). IMP was formed much more rapidly by the supernatant fraction than by the particulate fraction (Fig. 3c, f), indicating that this activity resides principally in the soluble fraction of the cell or is easily removed from the cytoplasmic membrane.

Fig. 3. The catabolism of 2.5 mM-[U- 14 C]AMP by the particulate (a, b, c) and supernatant (d, e, f) fractions of *Beneckea natriegens* in the absence of ATP (a, d), and in the presence of ATP at 5 mM (b, e) and 40 mM (c, f): ○, AMP; ●, adenosine; ▲, inosine; △, IMP. No results are shown for ribose 5-phosphate, hypoxanthine, adenine, and, where applicable, adenosine, IMP and inosine; for each of these compounds, counts were less than 10% of the total. Protein concentrations were (mg ml^{-1}): (a) 10, (b) 21, (c) 28, (d) 23, (e) and (f) 19.

With lower concentrations of ATP (5 and 10 mM), IMP was formed in cell-free extracts and then converted to inosine (Fig. 2b, c) due to a significant decrease in ATP and/or energy charge, or an increase in inorganic phosphate during the course of the experiment. IMP nucleotidase must therefore also be present in *B. natriegens*. Like AMP nucleotidase, this enzyme is probably membrane-bound: in the presence of 5 mM-ATP, [14 C]AMP was degraded only to IMP by the supernatant fraction, but with the particulate fraction AMP was converted first to IMP, then to inosine (Fig. 3b, e).

Though the above data suggest that AMP is converted to IMP by an ATP-activated AMP deaminase, it is possible that the pathway could be AMP $\rightarrow$ adenosine $\rightarrow$ inosine $\rightarrow$ IMP, with the last step being catalysed by inosine kinase (EC 2.7.1.73). The absence of detectable intermediates (adenosine and inosine) and the distributions of the activities in the particulate and supernatant fractions argue against this possibility. Moreover, when cell-free extracts were incubated with 2.5 mM-[U- 14 C]inosine in the presence of 40 mM-ATP, 70 to 77% of the 14 C remained as inosine after 30 min, and 10 to 15% was found as

Fig. 4. The effects of (a) ATP, (b) GTP, (c) UTP and (d) CTP (each at 40 mM) on the catabolism of 2.5 mM-[U- 14 C]AMP by cell-free extracts of *Beneckea natriegens*: ○, AMP; ▲, inosine; △, IMP. No results are shown for adenosine, ribose 5-phosphate, hypoxanthine, adenine, and, where applicable, inosine; for each of these compounds, counts were less than 6% of the total. Protein concentration was 16.5 mg ml $^{-1}$.

hypoxanthine. Less than 8% of the inosine was converted to IMP: therefore, inosine kinase is either absent or not active under these conditions.

If AMP is degraded to IMP by AMP deaminase, the phosphate group will be retained, but if the conversion is by AMP nucleotidase, adenosine deaminase and inosine kinase, then it will be lost and the γ -phosphate group of ATP will be introduced. When cell-free extracts, or particulate or supernatant fractions were incubated with 2.5 mM-[32 P]AMP in the presence of 40 mM-ATP, there was 87 to 94% conversion of the AMP to [32 P]IMP within 2 to 5 min. The retention of the labelled phosphate group in the resultant IMP is strong evidence for its direct formation by AMP deaminase.

AMP was only slowly degraded in the presence of GTP, UTP or CTP (Fig. 4), showing that these nucleotides inhibit AMP nucleotidase without significantly activating AMP deaminase. With GTP there was slow formation of IMP, but with UTP and CTP, IMP formation was followed by some inosine formation. Other AMP nucleotidases have previously been shown to be inhibited by purine and pyrimidine nucleoside triphosphates (Drummond & Yamamoto, 1971; Felicioli *et al.*, 1973; Ipata *et al.*, 1975).

DISCUSSION

AMP was catabolized by extracts of *B. natriegens*, in the absence of ATP, to adenosine by a membrane-bound AMP nucleotidase, followed by conversion to inosine by a soluble adenosine deaminase. ATP inhibited AMP nucleotidase and stimulated AMP deaminase, which was found in the supernatant fraction of cell extracts, enabling AMP to be converted to IMP. When relatively low levels of ATP were used, IMP was subsequently degraded to inosine by a particulate IMP nucleotidase as the ATP level fell during the course of the experiment.

Since many 5'-nucleotidases convert several ribonucleoside phosphates (Hurwitz *et al.*, 1957; Drummond & Yamamoto, 1971; Felicioli *et al.*, 1973; Ipata *et al.*, 1975), the AMP and IMP nucleotidase activities of *B. natriegens* possibly represent different functions of the same enzyme. AMP catabolism by extracts of *B. natriegens* is therefore remarkably similar to its catabolism by mammalian tissues (Burger & Lowenstein, 1967; Woods *et al.*, 1970; Drummond & Yamamoto, 1971; Chapman & Atkinson, 1973) and unlike its breakdown by AMP nucleosidase in *A. vinelandii* and *E. coli* (Schramm & Leung, 1973; Schramm & Lazorik, 1975).

To understand the metabolic function of the enzymes catalysing AMP breakdown, it is necessary to know their location within the bacterium. With cell sonicates of *B. natriegens* AMP deaminase was found in the supernatant fraction after centrifuging at 150 000 g and AMP nucleotidase was found in the particulate fraction (Fig. 3). The latter enzyme is thus firmly membrane-bound and probably not a periplasmic enzyme, unlike the AMP nucleotidase of *E. coli* and several other bacteria (Heppel, 1971). Since the AMP deaminase of *B. natriegens* is in the supernatant fraction it could be either a cytoplasmic enzyme or easily leached from the membrane, i.e. possibly periplasmic. Unfortunately, because *B. natriegens* is a marine bacterium, requiring Na⁺ and Mg²⁺ ions for cellular integrity, it is not possible to use osmotic shock techniques or to form sphaeroplasts using lysozyme/EDTA/sucrose to release periplasmic enzymes (cf. Heppel, 1971), since such treatments cause lysis.

At this stage one can only speculate why AMP is catabolized by these pathways in *B. natriegens*. Presumably either AMP nucleotidase or AMP deaminase is involved in regulation of the total adenine nucleotide content during growth, like rat liver AMP deaminase and *A. vinelandii* AMP nucleosidase (Chapman & Atkinson, 1973; Schramm & Leung, 1973; Knowles, 1977). However, *B. natriegens* is unlike *E. coli* and possibly other bacteria in that when there is a plentiful supply of substrate, but it is unable to grow due to depletion of the nitrogen source, there is a rapid increase in the intracellular adenine nucleotide pool size (Chapman *et al.*, 1971; Niven *et al.*, 1977; Knowles, 1977). This could be due either to an increased rate of *de novo* synthesis of AMP or, more likely, to a decreased net rate of adenine nucleotide consumption for macromolecular synthesis and an increased rate of salvage of AMP from RNA degradation. The ATP-activated AMP deaminase activity would be a method of controlling the adenine nucleotide content, or a way of regenerating scarce ammonia-nitrogen for other use. On the other hand, when the ATP pool size and energy charge fall due to starvation (Niven *et al.*, 1977), AMP could be degraded by the ATP-inhibited AMP nucleotidase in order to provide a carbon source and to maintain the energy charge.

To determine whether these speculations are valid, or whether there are other reasons for the pathways of AMP breakdown by *B. natriegens*, we are currently purifying the AMP catabolic enzymes to determine their catalytic and regulatory properties.

This investigation was supported by a grant from the Science Research Council. We wish to thank Dr Vern Schramm (Temple University) for helpful discussions and Dr Rick Martin (Cambridge University) for kindly supplying [^{32}P]AMP.

REFERENCES

- ATKINSON, D. E. (1968). The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. *Biochemistry* **7**, 4030–4034.
- BOOSMAN, A. & CHILSON, O. P. (1976). Subunit structure of AMP-deaminase from chicken and rabbit skeletal muscle. *Journal of Biological Chemistry* **251**, 1847–1852.
- BURGER, R. & LOWENSTEIN, J. M. (1967). Adenylate deaminase. III. Regulation of deamination pathways in extracts of rat heart and lung. *Journal of Biological Chemistry* **242**, 5281–5288.
- CHAPMAN, A. G. & ATKINSON, D. E. (1973). Stabilization of adenylate energy charge by the adenylate deaminase reaction. *Journal of Biological Chemistry* **248**, 8309–8312.
- CHAPMAN, A. G., FALL, L. & ATKINSON, D. E. (1971). Adenylate energy charge in *Escherichia coli* during growth and starvation. *Journal of Bacteriology* **108**, 1072–1086.
- DRUMMOND, G. I. & YAMAMOTO, M. (1971). Nucleotide phosphomonoesterases. In *The Enzymes*, vol. 4, 3rd edn, pp. 337–354. Edited by P. D. Boyer. London and New York: Academic Press.
- FELICIOLI, R. A., SENESI, S., MARMOCCHI, F., FALCONE, G. & IPATA, P. L. (1973). Nucleoside phosphomonoesterases during growth cycle of *Bacillus subtilis*. *Biochemistry* **12**, 547–552.
- GORNALL, A. G., BARDAWILL, C. J. & DAVID, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry* **177**, 751–766.
- HEPPEL, L. A. (1971). The concept of periplasmic enzymes. In *Structure and Function of Biological Membranes*, pp. 223–247. Edited by L. I. Rothman. London and New York: Academic Press.
- HURWITZ, J., HEPPEL, L. A. & HORECKER, B. L. (1957). The enzymatic cleavage of adenylic acid to adenine and ribose 5-phosphate. *Journal of Biological Chemistry* **226**, 525–540.
- IPATA, P. L., PALMARINI, C. A., SENESI, S., FALCONE, G. & FELICIOLI, R. (1975). The role of nucleoside triphosphates in the control of 3'- and 5'-AMP dephosphorylation in crude extracts of *B. subtilis*. *FEBS Letters* **57**, 195–197.
- KNOWLES, C. J. (1977). Microbial metabolic regulation by adenine nucleotide pools. *Symposia of the Society for General Microbiology* **27**, 241–283.
- KOCH, A. L. & VALLEE, G. (1959). The properties of adenosine deaminase and adenosine nucleoside phosphorylase in extracts of *Escherichia coli*. *Journal of Biological Chemistry* **234**, 1213–1218.
- KOHN, J. & REIS, J. L. (1963). Bacterial nucleotidases. *Journal of Bacteriology* **86**, 713–716.
- MAKAREWICZ, W. & STANKIEWICZ, A. (1976). Purification of AMP-deaminase from human skeletal muscle on 5'-AMP Sepharose 4B. *International Journal of Biochemistry* **7**, 245–247.
- NIVEN, D. F., COLLINS, P. A. & KNOWLES, C. J. (1977). Adenylate energy charge during batch culture of *Beneckea natriegens*. *Journal of General Microbiology* **98**, 95–108.
- SCHRAMM, V. L. & LAZORIK, F. C. (1975). The pathway of adenylate catabolism in *Azotobacter vinelandii*. Evidence for adenosine monophosphate nucleosidase as the regulatory enzyme. *Journal of Biological Chemistry* **250**, 1801–1808.
- SCHRAMM, V. L. & LEUNG, H. (1973). Regulation of adenosine monophosphate levels as a function of adenosine triphosphate and inorganic phosphate. A proposed metabolic role for adenosine monophosphate nucleosidase from *Azotobacter vinelandii*. *Journal of Biological Chemistry* **248**, 8313–8315.
- SMITH, H. O., KELLY, T. J. & ROY, P. H. (1974). Enzymatic methods for sequence analysis applied to DNA restriction and methylation sites. *Methods in Enzymology* **29**, 282–294.
- SMITH, L. D. & KIZER, D. E. (1969). Purification and properties of rat liver AMP deaminase. *Biochimica et biophysica acta* **191**, 415–424.
- WESTON, J. A. & KNOWLES, C. J. (1973). A soluble CO-binding c-type cytochrome from the marine bacterium *Beneckea natriegens*. *Biochimica et biophysica acta* **305**, 11–18.
- WESTON, J. A. & KNOWLES, C. J. (1974). The respiratory system of the marine bacterium *Beneckea natriegens*. I. Cytochrome composition. *Biochimica et biophysica acta* **333**, 228–236.
- WOODS, H. F., EGGLESTON, L. V. & KREBS, H. A. (1970). The cause of hepatic accumulation of fructose-1-phosphate on fructose loading. *Biochemical Journal* **119**, 501–510.
- YAGIL, E. & BEACHAM, I. R. (1975). Uptake of adenosine 5'-monophosphate by *Escherichia coli*. *Journal of Bacteriology* **121**, 401–405.
- YATES, M. G. (1969). A non-specific adenine nucleotide deaminase from *Desulfovibrio desulfuricans*. *Biochimica et biophysica acta* **171**, 299–310.