Biochimica et Biophysica Acta, 389 (1975) 145–153

BBA 76924

ACTION OF CYTOCHALASIN A, A SULFHYDRYL-REACTIVE AGENT, ON SUGAR METABOLISM AND MEMBRANE-BOUND ADENOSINE TRIPHOSPHATASE OF YEAST

S.-C. KUO and J. OLIVER LAMPEN

Waksman Institute of Microbiology, Rutgers University, The State University of New Jersey, New Brunswick, N. J. 08903 (U.S.A.)

(Received October 21st, 1974)

SUMMARY

Cytochalasin A at 10–20 μg/ml inhibits growth and sugar uptake by *Saccharomyces* strain 1016. The effects of cytochalasin A in intact cells were completely prevented when 1 mM cysteine or dithiothreitol was added along with cytochalasin A, but were not eliminated by thiols added after inhibition had occurred. Purified yeast hexokinase, glucose-6-*P* dehydrogenase, phosphofructokinase and aldolase were not sensitive to cytochalasin A (20 μg/ml). Glyceraldehyde-3-*P* dehydrogenase was strongly inhibited by cytochalasin A (5 μg/ml); activity was promptly restored by thiols. Anaerobic glycolysis was inhibited by cytochalasin A or by iodoacetate; unlike iodoacetate, cytochalasin A did not cause accumulation of sugar phosphates. In contrast, cytochalasin A, but not iodoacetate, inhibited isolated membrane-bound ATPases. Cytochalasin A is a sulfhydryl-reactive agent and has membrane-related effects (adenosine triphosphatase) which may well be the basis of its interference with energy-dependent uptake of solutes.

INTRODUCTION

The cytochalasins are a group of mold metabolites that exert an inhibitory effect on a variety of cell functions [1–4]. Although cytochalasin A was first detected by virtue of the unusual morphological effects it produced in fungal hyphae [5], most studies have dealt with the action of cytochalasin B on cultured mammalian cells. Recently cytochalasin B has been reported to be a potent, reversible inhibitor of sugar transport in mammalian cells [6, 7]. Cytochalasin A inhibited sugar uptake (transport plus phosphorylation) in chick embryo fibroblasts, but was less potent, and the inhibition was less readily reversible than that produced by cytochalasin B [4].

In contrast to mammalian cells, bacteria, yeasts and fungi are sensitive to cytochalasin A but not to cytochalasin B [8–10]. Cytochalasin A inhibited the energy-

Abbreviation: DCCD, *N,N'*-dicyclohexylcarbodiimide.

dependent uptake (and accumulation) of metabolizable sugars, amino acids, and nucleotides and of the non-metabolizable amino acid analogue, α-aminoisobutyric acid by intact cells of *Saccharomyces* strain 1016, while the transport of non-metabolizable hexoses by carrier-mediated facilitated diffusion was unaffected [10].

We report here that cytochalasin A is a sulfhydryl-reactive agent, since its inhibitory effects on yeast can be abolished by cysteine or dithiothreitol. In addition, it inhibits in vitro, the membrane-bound adenosine triphosphatases (ATPases) from yeast. The implications of these findings with respect to the inhibition of energy-dependent reactions in yeast cells are discussed.

MATERIALS AND METHODS

Growth of organisms. Saccharomyces strain 1016 was grown in modified Vogel's medium N with 0.2 M glucose as the carbon source [11]. To examine its inhibitory effect on growth, cytochalasin A was added to exponential phase cells; growth was followed by measuring the turbidity with a Klett-Summerson colorimeter (filter no. 66). A reading of 120 corresponds to about $4 \cdot 10^7$ cells of strain 1016 per ml.

Estimation of glucose uptake. The washed cells were suspended in fresh growth medium at about $3 \cdot 10^7$ per ml. [^{14}C]Glucose (10 mM; 0.1 μCi/ml) was added after the cells had been preincubated with cytochalasin A for 10 min. At various intervals samples were withdrawn, the cells collected on Whatman GF/A filter paper, washed with 10 ml ice-cold water and the radioactivity counted as previously described [11].

Preparation of the crude "mitochondria" and "plasma membrane" fractions from lysed protoplasts. Cells were grown in 0.3 % yeast extract/0.5 % Bactopeptone medium containing 0.2 M glucose at 30 °C and harvested in exponential growth phase, spun down at 1 000 × *g* for 3 min and washed twice with glass distilled water. The washed cells were suspended at $5 \cdot 10^8$ per ml in 40 ml of 1.0 M sorbitol solution containing 10 mM Tris/maleate buffer (pH 6.0), 2 mM $MgCl_2$, 15 mM β-mercaptoethanol and 1.5 ml Glusulase (Endo Laboratories). After 60 min incubation more than 90 % of cells had been converted to protoplasts. The protoplasts were then sedimented at 4 000 × *g* for 5 min and washed twice with 1.0 M sorbitol solution. The plasma membrane fraction was separated from mitochondrial membranes as follows. Protoplasts were suspended in 0.4 M sorbitol solution to protect the integrity of the mitochondria [12, 13] and the partially lysed protoplasts were homogenized with 10 strokes of a Kontes homogenizer. Approx. 80–90 % of the protoplasts were lysed by this procedure. The homogenate was centrifuged at 2500 × *g* for 5 min to remove cell debris and the supernatant fluid was then spun at 9 500 × *g* for 10 min. The sediment, containing mitochondria (high alcohol dehydrogenase and NADH oxidase activity) and some large pieces of membrane, was washed twice with 0.4 M sorbitol solution. The supernatant fluid containing small pieces of plasma membrane, was centrifuged at 32 000 × *g* for 20 min and washed twice with 0.4 M sorbitol solution to yield the plasma membrane fraction.

Anaerobic glycolysis and levels of intracellular sugar intermediates. Cells were grown overnight in an atmosphere of N_2, washed twice with glass distilled water and suspended in 20 mM phosphate buffer, pH 7.0, with 40 mM glucose at $2 \cdot 10^8$ cells per ml. The suspension was incubated at 30 °C under N_2. After equilibration

for 20 min the cell suspension was divided into three portions and incubated with or without added drug. At 0 time and after 40 min of incubation, samples (5 ml) were removed and spun down at 3 000 $\times g$ for 2 min. The clear supernatant fluids were collected for measurement of residual glucose, and 2 ml of 5 % ice-cold $HClO_4$ was added to the pellets to stop the reaction and to extract the phosphate esters [11].

Sugar phosphates and glyceraldehyde 3-phosphate were assayed by the formation of NAD, NADH or NADPH in systems containing NADH, NAD or NADP and glucose-6-phosphate dehydrogenase (EC 4.1.1.49) (for glucose-6-*P*); aldolase (EC 4.1.2.13), triosephosphate isomerase (EC 5.3.1.1) and glycerol-1-phosphate dehydrogenase (EC 1.1.99.5) (for fructose-1, 6-P_2); and glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (for glyceraldehyde-3-*P*). Intracellular concentrations were calculated according to the method described by Cirillo [14].

Enzyme assays. Glyceraldehyde-3-phosphate dehydrogenase activity was measured at room temperature by following the increase in absorbance at 340 nm accompanying the reduction of NAD resulting from glyceraldehyde 3-phosphate oxidation. The reaction mixture (1.0 ml) consisted of 80 μmol of Tris · HCl buffer, pH 8.5, 17 μmol of Na_2HAsO_4, pH 8.5, 20 μmol of NaF, 1 μmol of NAD, 1.2 μmol of DL-glyceraldehyde 3-phosphate, and glyceraldehyde-3-phosphate dehydrogenase (Sigma, 15 μg of protein).

ATPase activity was determined at 30 °C in a medium containing 20 μmol of Tris · HCl at pH 7.5 (for 32 000 $\times g$ pellet) or pH 8.5 (9 500 $\times g$ pellet), 2.5 μmol of $MgCl_2$, 2.5 μmol of ATP, and membrane protein (50–100 μg) in a final volume of 1.0 ml. The reaction was terminated by the addition of 1.0 ml of 10 % $HClO_4$. After centrifugation the clear supernatant fluid was removed for phosphate analysis as described by Ernster et al. [15]. The value was corrected for non-enzymatic degradation of the ATP and for the liberation of P_i in the absence of ATP. The enzyme activities were expressed as nmol of P_i formed per min mg of protein. Protein was measured by the method of Lowry et al. [16].

Chemicals. All chemicals were reagent grade. Hexokinase (EC 2.7.1.1), phosphofructokinase (EC 2.7.1.11), glucose-6-phosphate dehydrogenase, aldolase, triphosphate isomerase and glycerol-l-phosphate dehydrogenase were purchased from Boehringer Mannheim Co., Germany. Glyceraldehyde-3-phosphate dehydrogenase (yeast), *N,N'*-dicyclohexylcarbodiimide (DCCD), oligomycin, ATP, NAD, NADH, NADPH and dithiothreitol were from Sigma Chemical Co.; iodoacetic acid from Eastman Kodak Co.; [U-^{14}C]glucose from New England Nuclear Corp; cytochalasin A and cytochalasin B from Imperial Chemicals, England or from Aldrich Chemical Co. Cytochalasin A and cytochalasin B were stored at 4 °C as 5–10 mg/ml stock solutions in dimethylsulfoxide.

RESULTS AND DISCUSSION

Prevention by thiols of the inhibition of growth and glucose uptake by cytochalasin A

We have reported [10] that cytochalasin A at 10–20 μg per ml halted growth and sugar uptake by *Saccharomyces* strain 1016 almost completely in 1–2 h. Subsequently we observed that the inhibition of intact cells by cytochalasin A could be completely prevented when 1 mM cysteine or dithiothreitol was added at the same time as the drug (Figs 1A and 1B). If addition of cysteine was delayed until 30 min

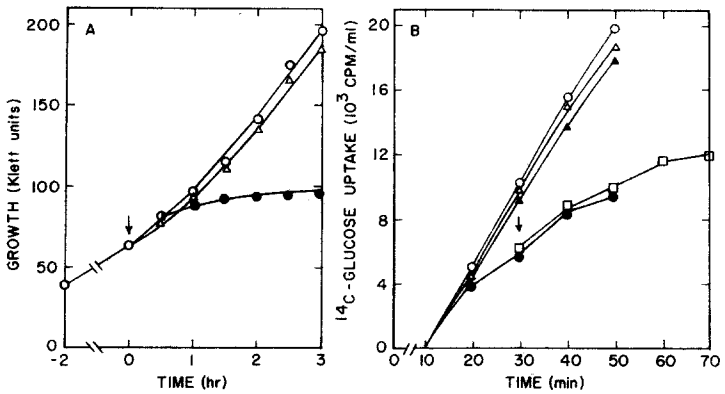

Fig. 1. Inhibition of the growth (A) and uptake of [^{14}C]glucose (B) of *Saccharomyces* strain 1016 by cytochalasin A in the presence and absence of cysteine or dithiothreitol. (A) The cells were grown at 30 °C in modified Vogel's medium with 0.1 M glucose. After 2 h incubation, the culture was divided into three portions which were supplemented (↓) as indicated. Cytochalasin A was added as a solution in dimethylsulfoxide. The final concentration of dimethylsulfoxide was 0.5 % in all flasks. Symbols: ○, control; ●, cytochalasin A (20 μg/ml); △, cytochalasin A plus cysteine (1 mM). (B) Cells were washed with water, suspended in fresh medium at 3×10^7/ml, and incubated with cytochalasin A and thiols as indicated. After 10 min, [U-^{14}C]glucose (10 mM; 0.1 μCi/ml) was added and the uptake was measured as described in Materials and Methods. Symbols: ○, control; ●, cytochalasin A (20 μg/ml); △, cytochalasin A plus cysteine (1 mM); ▲, cytochalasin A plus dithiothreitol (1 mM); □, cysteine (1 mM) added (↓) after 30 min of cytochalasin A treatment.

after the cytochalasin A, the uptake of sugar was no longer protected (Fig. 1B). Under the conditions employed, cysteine or dithiothreitol had no effect in the absence of cytochalasin A. These observations are in accord with the findings by Haslam [17] that platelet-clot retraction was inhibited by cytochalasin A and that cysteine blocked this effect; hence that cytochalasin A is a sulfhydryl-reactive agent. On the other hand, Cu^{2+} (0.1 mM) or Hg^{2+} (2 μM) salts potentiated the inhibitory activity of cytochalasin A on intact cells (data not presented in detail). These ions probably interact with sulfhydryl groups on the yeast plasma membrane [18] which otherwise would bind with and inactivate the cytochalasin A. The lack of reversibility in vivo by thiols suggest that cytochalasin A reacts with sulfhydryl groups on intracellular components as well as with those on the membrane. Another possibility is that it specifically interacts with a critical non-sulfhydryl membrane component(s).

Effect on glycolytic enzymes in vitro

It was of interest to see if the inhibition of sugar uptake resulted from an effect of cytochalasin A on the enzymes of the glycolytic pathway and thus on the generation of ATP. Purified yeast hexokinase, glucose-6-*P* dehydrogenase, phosphofructokinase, and aldolase were not sensitive to cytochalasin A levels as high as 20–30 μg per ml (data not presented in detail). Glyceraldehyde-3-*P* dehydrogenase (yeast) was strongly inhibited by cytochalasin A at 5 μg per ml (Table I); at 10 and 20 μg per ml inhibition was 85 and 93 %, respectively. Activity was promptly restored by subsequent addition of 1 mM cysteine or dithiothreitol (Fig. 2). Iodoacetate, a sulfhydryl-reactive agent, also severely inhibited enzyme activity. On the other hand, cytochala-

TABLE I

SENSITIVITY OF GLYCERALDEHYDE-3-*P* DEHYDROGENASE TO CYTOCHALASIN A, CYTOCHALASIN B AND IODOACETATE

Yeast glyceraldehyde-3-phosphate dehydrogenase (15 μg protein) was incubated at room temperature with the indicated additions in a final volume of 100 μl of 100 mM Tris · HCl, pH 8.5. At the times shown, 20-μl samples were removed, added to the assay mixture and the activity measured as described in Materials and Methods. Dimethylsulfoxide at 0.5 % was included in all experiments. Cytochalasin A, 5 μg/ml is equivalent to 10.5 μM.

Pretreatment	Time (min)	Relative activity (%)
None		100
Cytochalasin A		
5 μg/ml	5	50
5 μg/ml	10	25
10 μg/ml	10	15
20 μg/ml	10	7
Cytochalasin B, 20 μg/ml	10	103
Iodoacetate, 1 mM	5	68
	10	25

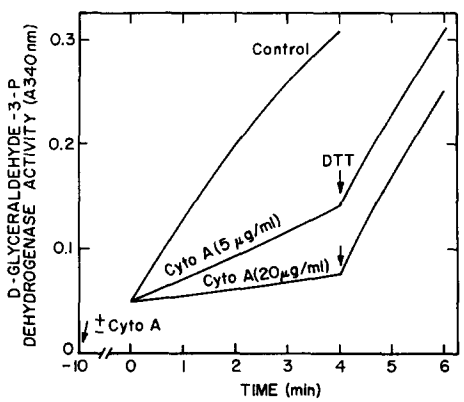

Fig. 2. Inhibition of glyceraldehyde-3-phosphate dehydrogenase by cytochalasin A and its annulment by dithiothreitol. The enzyme was preincubated for 10 min without or with cytochalasin A (concentration indicated in the figure) in a final volume of 100 μl, as in the legend of Table I. At 0 min 20-μl samples were removed, added to the assay mixture and the activity measured by the increase in absorbance at 340 nm with a Beckman DB spectrophotometer as described in Materials and Methods. Dithiothreitol (1 mM) was added at the time indicated (↓).

sin, B, which is inactive against yeast growth, did not affect glyceraldehyde-3-*P* dehydrogenase.

Accumulation of sugar phosphates

Iodoacetate is a rather specific inhibitor of anaerobic glycolysis in yeast. It selectively blocks glyceraldehyde-3-*P* dehydrogenase and under appropriate condi-

TABLE II

EFFECT OF CYTOCHALASIN A AND IODOACETATE ON INTRACELLULAR LEVELS OF SUGAR PHOSPHATES

Washed cells from anaerobically grown cells were suspended at 2×10^8/ml in 20 mM phosphate buffer, pH 7.0, with 40 mM glucose and incubated at 30 °C under N_2. After equilibration for 20 min, cytochalasin A or iodoacetate was added and incubation continued for 40 min. Intracellular sugar phosphates and glyceraldehyde 3-phosphate were determined as described in Materials and Methods. Extracellular glucose was determined with glucose oxidase.

Time	External glucose (mM)	Glucose-6-*P* (mM)	Fructose-6-*P* (mM)	Fructose-1,6-P_2 (mM)	Glyceraldehyde-3-*P* (mM)
−20 min	40	0.33	<0.05	1.90	<0.05
0 min	30	0.33	<0.05	1.83	<0.05
+40 min control	1.5	0.31	<0.05	0.77	<0.05
Cytochalasin A, 40 μg/ml	12.4	0.50	<0.05	0.83	<0.05
Iodoacetate, 2 mM	12.0	2.14	<0.05	3.82	<0.05

tions causes the accumulation of fructose-1, 6-P_2 and glucose-6-*P* [19]. The effect of cytochalasin A on anaerobic glycolysis and on the level of intracellular sugar phosphate intermediates is demonstrated in Table II. Anaerobically grown cells were suspended in fresh medium with 40 mM glucose and incubated under N_2, either in the absence of an inhibitor or in the presence of cytochalasin A or iodoacetate. The glucose concentration dropped from 40 to 30 mM during the first 20 min of incubation; the level of intracellular sugar phosphates did not change under these conditions. The concentrations of fructose-6-*P* and glyceraldehyde-3-*P* were less than 0.05 mM. After incubation for 40 min, glucose had been almost completely consumed in the control culture and the level of fructose-1, 6-P_2 had decreased to about 50 % of the original value. In the presence of cytochalasin A or iodoacetate, glucose utilization was severely inhibited (approx. 12 mM glucose remained after 40 min). The intracellular levels of sugar phosphates and glyceraldehyde-3-*P* were not significantly affected by treatment with cytochalasin A, whereas iodoacetate caused a large accumulation of both glucose-6-*P* and fructose-1, 6-P_2 (Table II). We conclude from these results that blockage of glyceraldehyde-3-*P* oxidation is probably not important to the action of cytochalasin A in vivo, even though that enzyme is highly sensitive to the drug in vitro.

Effect on isolated membrane-bound ATPases

Since no accumulation of glucose-6-*P* or fructose-1, 6-P_2 occurred in the cells treated with cytochalasin A (Table II), the rapid depletion of intracellular ATP and the cessation of energy-dependent accumulation of solutes in the presence of the drug [10] may result from an alteration of membrane function. The effect of cytochalasin A on the activity of membrane-bound ATPases was thus examined. There are at least two ATPase species in yeast, one in the mitochondria, the other in the plasma membrane [20–22]. In our studies, the plasma membrane fraction (32 000 × *g* pellet) was prepared from protoplasts homogenized in 0.4 M sorbitol medium as described in Materials and Methods; the mitochondria remained relatively intact

TABLE III

EFFECT OF INCUBATION WITH CYTOCHALASIN A AND VARIOUS INHIBITORS ON MEMBRANE-BOUND ATPase ACTIVITY FROM YEAST

Membrane fractions prepared as described in Materials and Methods; 70 μg protein per ml was subsequently used in the assay mixture for ATPase activity.

Treatment	ATPase activity			
	9500 × *g* pellet		32 000 × *g* pellet	
	Expt 1	Expt 2	Expt 1	Expt 2
Control	443* (100)**	429 (100)	143 (100)	150 (100)
Cytochalasin A				
10 μg/ml	358 (81)	—	100 (70)	—
20 μg/ml	279 (63)	300 (70)	71 (50)	65 (44)
30 μg/ml	229 (52)	229 (53)	57 (39)	46 (31)
40 μg/ml	172 (39)	—	43 (30)	—
Cytochalasin B				
40 μg/ml		400 (93)		146 (98)
Oligomycin,				
10 μg/ml		107 (25)		71 (48)
20 μg/ml		86 (20)		64 (46)
Control		436 (100)		164 (100)
DCCD, 20 mM		193 (44)		93 (57)
Iodoacetate,				
100 μM		409 (93)		157 (96)

* nmol P_i/min per mg protein.
** Percent of control value.

under these conditions and were collected as a 9500 × *g* pellet [12, 13]. The activities of alcohol dehydrogenase and NADH oxidase (both mitochondrial enzymes) were found to be several-fold higher in the 9500 × *g* than in the 32 000 × *g* fraction (data not presented in detail). The ATPase activities from both preparations were activated by Mg^{2+}, but their pH optima and oligomycin sensitivity differed (see Table III). ATPase activity from the 9500 × *g* pellet has its optimum between pH 8 and 9, whereas, the optimum for the 32 000 × *g* pellet is at pH 7.5. These results are consistent with the report by Fuhrmann et al. [22]. It may be concluded that the 32 000 × *g* fraction contains mostly plasma membrane, whereas the 9500 × *g* fraction consists of mitochondria and some large pieces of plasma membrane. The results in Table III demonstrate that both membrane-bound ATPases are inhibited by cytochalasin A (but not iodoacetate), with the 32 000 × *g* fraction being somewhat more sensitive than the 9500 × *g* fraction. At 40 μg cytochalasin A per ml (84 μM), there is 60–70 % inhibition of the ATPase activities. Since only the crude membrane preparations have been examined in these studies and cytochalasin A may interact with other membrane components, it is difficult to correlate quantitatively the inhibition of ATPase and that of growth and of sugar uptake (Fig. 1). Cytochalasin B has no effect on either yeast ATPase; however, in mammalian systems the Ca^{2+}- or Mg^{2+}-stimulated ATPase of actomyosin and myosin are sensitive to cytochalasin B and cytochalasin D [23–25].

DCCD has been shown to inhibit Mg^{2+}-activated membrane-bound ATPases from bacteria and beef heart [26–29]. The ATPases of the yeast mitochondrial and plasma membrane fractions were equally sensitive to DCCD, i. e. about 50 % inhibition with 20 μM DCCD (Table III) and 90 % with 100 μM DCCD. The Mg^{2+}-activated ATPases of microorganisms are reported to be involved in solute transport by coupling reversibly the hydrolysis of ATP to the translocation of protons through the membrane [29–31]. In *Saccharomyces*, the proton movements appear intimately linked to active transport of amino acids and disaccharides, but not of hexoses [32, 33]. The role of the plasma membrane-bound ATPase of yeast in energy-dependent uptake of solutes has not been studied, but it may resemble that of the bacterial or mitochondrial enzymes since all of them require Mg^{2+} and are sensitive to DCCD (Table III; [28, 29]).

From our results it is clear that cytochalasin A will react readily with sulfhydryl groups. There is no direct indication that this property is significant for its inhibitory action since cytochalasin A inhibition of sugar uptake could be prevented but not annulled by thiols (Fig. 1). Nevertheless, it is possible that the critical binding involves sulfhydryl groups embedded in the membrane or on its interior surface and inaccessible to external thiols. To explain the observed impairments of energy-dependent processes [10] we tentatively suggest that cytochalasin A interacts with the membrane-bound Mg^{2+}-ATPase of the yeast cell and in this way interferes with energy coupling and ATP regeneration. Cytochalasin A may prove valuable for determination of the precise role of the membrane ATPases in these processes.

ACKNOWLEDGMENTS

We thank Mary Anne Talle for her excellent technical assistance. This work was supported by Public Health Service grant AI-04572 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

1 Carter, S. B. (1967) Nature 213, 261–264
2 Carter, S. B. (1972) Endeavor 31, 77–82
3 Cohn, R. H., Banerjee, S. D., Shelton, E. R. and Bernfield, M. R. (1972) Proc. Natl. Acad. Sci. U.S. 69, 2865–2869
4 Kletzien, R. F., Perdue, J. F. and Springer, A. (1972) J. Biol. Chem. 247, 2964–2966
5 Aldridge, D. C., Armstrong, J. J., Speake, R. N. and Turner, W. B. (1967) Chem. Commun. 1, 26–27
6 Zigmond, S. H. and Hirsch, J. G. (1972) Science 176, 1432–1434
7 Kletzien, R. F. and Perdue, J. F. (1973) J. Biol. Chem. 248, 711–719
8 Wessells, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E. L., Wrenn, J. T. and Yamada, K. M. (1971) Science 171, 135–143
9 Betina, V., Micekova, D. and Nemec, P. (1972) J. Gen. Microbiol. 71, 343–349
10 Kuo, S.-C. and Lampen, J. O. (1974) Ann. N.Y. Acad. Sci. 235, 137–148
11 Kuo, S.-C. and Lampen, J. O. (1972) J. Bacteriol. 111, 419–429
12 Duell, E. A., Inoue, S. and Utter, M. F. (1964) J. Bacteriol. 88, 1762–1773
13 Ohnishi, T., Kawaguchi, K. and Hagihara, B. (1966) J. Biol. Chem. 241, 1797–1806
14 Cirillo, V. P. (1962) J. Bacteriol. 84, 485–491
15 Ernster, L., Zetterstrom, R. and Lindberg, O. (1950) Acta Chem. Scand. 4, 942–947
16 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275

17 Haslam, R. J. (1972) Biochem. J. 127, 34P
18 Rothstein, A. (1970) in Current Topics in Membranes and Transport (Bronner, F. and Kleinzeller, A., eds), Vol. 1, pp. 135–176, Academic Press, Inc., New York
19 Webb, J. L. (1966) in Enzyme and Metabolic Inhibitors (Webb, J. L., ed.), Vol. III, pp. 61–86, Academic Press, Inc., New York
20 Matile, Ph., Moor, H. and Muhlethaler, K. (1967) Arch. Microbiol. 58, 201–211
21 Tzagoloff, A. (1970) J. Biol. Chem. 245, 1545–1551
22 Fuhrmann, G. F., Wehrli, E. and Boehm, C. (1974) Biochim. Biophys. Acta 363, 295–310
23 Spudnich, J. A. and Lin, S. (1972) Proc. Natl. Acad. Sci. U.S. 69, 442–446
24 Puszkin, E., Puszkin, S., Lo, L. W. and Tannenbaum, S. W. (1973) J. Biol. Chem. 248, 7754–7761
25 Nicklas, W. J. and Berl, S. (1974) Nature 247, 471–473
26 Holloway, C. T., Robertson, A. M., Knight, I. G. and Beechey, R. B. (1966) Biochem. J. 100, 79P
27 Bulos, B. and Racker, E. (1968) J. Biol. Chem. 243, 3891–3900
28 Harold, F. M., Baarda, J. R., Baron, C. and Abrams, A. (1969) J. Biol. Chem. 244, 2261–2268
29 Harold, F. M. (1972) Bacteriol. Rev. 36, 172–230
30 Asghar, S. S., Levin, E. and Harold, F. M. (1973) J. Biol. Chem. 248, 5225–5233
31 Mitchell, P. and Moyle, J. (1974) Biochem. Soc. Trans. 2, 463–466
32 Eddy, A. A. and Nowacki, J. A. (1971) Biochem. J. 122, 701–711
33 Seaston, A., Inkson, C. and Eddy, A. A. (1973) Biochem. J. 134, 1031–1043