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INVOLVEMENT OF PHOSPHATE-MODIFIED ATP ANALOGS IN THE REACTIONS OF OXIDATIVE PHOSPHORYLATION

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Summary

Various analogs of adenosine 5′-triphosphate with a modified terminal phosphate group have been tested in energy-requiring reactions with intact mitochondria and submitochondrial particles.

It is shown that the fluorophosphate analog ATP(γF) is a strong inhibitor of mitochondrial respiration and of energy requiring reactions which involve the participation of high energy intermediates, generated aerobically by the respiratory chain. On the other hand, ATP(γF) does not affect the ATPase activity of intact or disrupted mitochondria and is less effective in inhibiting ATP-driven reactions.

The imidophosphate analog AMP-*P*(NH)*P* also inhibits the partial reactions of oxidative phosphorylation, but does not affect ATP synthesis from ADP and P_i. In contrast to ATP(γF), it is a strong inhibitor of both soluble and membrane-bound mitochondrial ATPases.

The biological implication of the complementary effects of ATP(γF) and AMP-*P*(NH)*P* on mitochondria-catalysed reactions is discussed while suggesting the use of such nucleotide analogs as specific tools for the study of ATP-forming and ATP-utilizing reactions in mitochondria.

Abbreviations: ATP(γF), adenosine 5′-(3-fluoro)triphosphate; ATP(γMe), adenosine 5′-(3-*O*-methyl)-triphosphate; ATP(γPh), adenosine 5′-(3-*O*-phenyl)triphosphate; GTP(γF), guanosine 5′-(3-fluoro)triphosphate; AMP-*P*(NH)*P*, 5′-adenylyl-(β,γ-imido)diphosphate.

Introduction

Recent progress in the preparative chemistry of nucleotides has led to the availability of a large number of synthetic ATP analogs with specific modifications in the purine ring, in the ribose moiety or in the polyphosphate chain (Refs. 1—3 and references therein). Due to the involvement of adenine nucleotides in virtually all energy-producing and energy-utilizing reactions, adenine nucleotide analogs have become particularly useful tools for probing the mechanisms of ATP synthesis and breakdown by whole mitochondria and/or submitochondrial particles [4—14].

While investigating the participation of various base-modified ATP analogs in the reactions of oxidative phosphorylation, we have shown that such analogs were unable to penetrate the intact mitochondrial membrane and could not be used as substrates by the respiratory chain enzymes [15—17]. However, the base-modified ATP analogs were able to participate in transphosphorylation reactions, being good substrates for mitochondrial phosphotransferases located in the intermembrane space [18,19].

In the present study we now describe the participation of phosphate-modified adenine nucleotides in the reactions catalysed by intact mitochondria and/or submitochondrial particles.

Materials and Methods

All adenine nucleotides and nicotinamide adenine dinucleotides, phospho*enol*pyruvate and AMP-*P*(NH)*P* were commercial products from Boehringer Mannheim, F.R.G. The synthesis and purification of the modified nucleotides ATP(γF), ATP(γMe), ATP(γPh) and GTP(γF) was performed according to procedures previously published [20—22]. The ^{14}C-labeled adenine nucleotides were purchased from the Radiochemical Centre, Amersham, U.K. The following enzymes were also products of Boehringer, Mannheim: hexokinase (EC 2.7.1.1), pyruvate kinase (EC 2.7.1.40), adenylate kinase (EC 2.7.4.3), nucleosidediphosphate kinase (EC 2.7.4.6), glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30). Mitochondria from rat and guinea pig liver were isolated as previously described [18,23]. Mitochondria from rat brain were isolated in a mannitol/sucrose/EDTA/albumin medium, essentially as described for the preparation of those from rat pancreas [24]. Protein concentrations were measured by the method of Lowry et al. [25]. Rat liver submitochondrial 'lubrol particles' were obtained by the procedure of Chan et al. [26], as modified by Bârzu et al. [17]. Other analytical procedures, such as measurement of mitochondrial respiration, the exchange of intramitochondrial ^{14}C-labeled adenine nucleotides with externally added nucleotides and the determination of individual enzyme activities was performed as described in preceding papers [15,16,18]. The rate of ATP synthesis by 'lubrol particles' was measured from the amount of NADPH formed in the presence of an excess of glucose, hexokinase, glucose-6-phosphate dehydrogenase and $NADP^+$ [7]. In separate experiments we also checked whether the activity of the coupling enzymes was affected by the nucleotides under investigation. The energy-linked reduction of intramitochon-

drial NAD^+ by succinate was assayed fluorimetrically or by measuring the reduction of acetoacetate to β-hydroxybutyrate, as described by Ernster and Lee [27]. Additional experimental details are given in the legends to the figures.

Results and Discussion

Effects on the respiratory activity of intact mitochondria

Of the investigated ATP derivatives with a modified terminal phosphate group, the fluorophosphate analog ATP(γF) showed the most interesting effect on the respiratory activity of intact mitochondria. The addition of ATP(γF) to respiring rat brain mitochondria results in a progressive decline of the respiratory rate in State 3, leaving State 4 respiration unaffected. This inhibitory effect of ATP(γF) is not immediate, similar to that of oligomycin and octylguanidine [28,29]; the degree of inhibition increases with incubation time and is strongly dependent on the source of mitochondria. Most sensitive are rat brain mitochondria, for which half-maximal inhibition of State 3 respiration (at the third ADP addition) is reached at 60 μM ATP(γF). With rat liver mitochondria the same effect is achieved at an ATP(γF) concentration of 300 μM, while guinea pig liver mitochondria are practically insensitive to inhibition by ATP(γF). The inhibition of mitochondrial respiration by ATP(γF) does not depend on whether the ATP analog is added at State 3 or at State 4; it is independent of the respiratory substrate and can be released by 2,4-dinitrophenol, although not entirely. The time-dependent inhibition of respiration requires that ADP is phosphorylated; previous incubation of mitochondria with ATP(γF) in the absence of ADP does not increase the degree of inhibition. The inhibition cannot be counteracted by ATP or other nucleotides, as exemplified in Table I for a combination of ATP(γF) and AMP-*P*(NH)*P*, where the latter does not interfere with the inhibitory effect of ATP(γF).

To explain the progressive inhibition of the ADP-released respiration by ATP(γF), one could invoke either a slow penetration of the adenine analog into the inner mitochondrial compartment, or a slow covalent reaction between the catalytic site of F_1 and ATP(γF). Conceivably, ATP(γF) could inhibit the mitochondrial respiration by acting either at the translocase site or at the site involving the synthesis of ATP from ADP and P_i, or at both. In order to distinguish between these possibilities, we have investigated the effect of ATP(γF) on the translocation of ^{14}C-labeled ADP. At a [^{14}C]ADP concentration of 50 μM, the transport of labeled adenine nucleotides was monitored in the presence of increasing amounts of ATP(γF), up to a ten-fold excess of the latter. At 500 μM ATP(γF), the incorporation of radioactive ADP into mitochondria was reduced by only 28%, indicating that there is no considerable inhibition of mitochondrial respiration at the ADP-ATP translocase level.

In order to establish whether the inhibitory effect of ATP(γF) can be associated with a modified terminal phosphate group, we have tested other analogs with modifications in the γ-position of the polyphosphate chain such as ATP(γMe) and ATP(γPh) or with a modified bridge between the β- and γ-phosphate group such as AMP-*P*(NH)*P*. It is evident from the results in Table I that, unlike ATP(γF), the latter nucleotide analogs are unable to interfere with

TABLE I

EFFECT OF PHOSPHATE-MODIFIED ATP ANALOGS ON THE RESPIRATORY ACTIVITY OF INTACT RAT BRAIN MITOCHONDRIA

The basic respiratory medium contained in a final volume of 0.5 ml: 180 mM sucrose, 25 mM Tris-HCl (pH 7.0), 50 mM KCl, 5 mM potassium phosphate (pH 7.4), 2.5 mM $MgCl_2$, 1 mM EDTA, 5 mM glutamate, 1 mg defatted bovine serum albumin. After addition of rat brain mitochondria (0.9—1.2 mg or protein), ADP was injected in three successive steps. For each ADP addition, State 3, State 4 and the ADP/O ratio were calculated. The results in this table represent the mean value from three separate experiments using different mitochondrial preparations. n.d., not determined.

Nucleotides	ngatoms O/min per mg protein		State 3/ State 4	ADP/O
	State 3	State 4		
Control	58.8	11.8	4.98	2.70
0.04 mM ATP(γF)				
First ADP addition (66 nmol)	48.8	10.7	4.56	2.65
Second ADP addition (132 nmol)	42.7	12.2	3.46	2.65
Third ADP addition (132 nmol)	36.6	12.8	2.86	2.58
0.30 mM ATP(γP)				
First ADP addition (66 nmol)	41.5	11.3	3.67	2.60
Second ADP addition (132 nmol)	25.6	12.2	2.10	2.44
Third ADP addition (132 nmol)	19.5	12.4	1.57	n.d.
ATP(γMe) (1 mM)	54.7	12.2	4.48	2.52
ATP(γPh) (1 mM)	56.7	12.3	4.61	2.62
AMP-*P*(NH)P(1 mM)	59.0	13.0	4.54	2.60
0.1 mM ATP(γP) + 1 mM AMP-*P*(NH)*P*				
First ADP addition (66 nmol)	46.2	10.9	4.22	2.63
Second ADP addition (132 nmol)	33.2	11.4	2.91	2.55
Third ADP addition (132 nmol)	20.6	11.3	1.82	2.48

the respiratory activity of intact mitochondria even at concentrations as high as 1 mM. These experimental results leave considerable room for speculation on possible structure vs. function correlations. However, it is not our intention at this stage to theorize on such correlations, although it is worthwhile mentioning at least one factor which seems to be critical, the size of the terminal phosphate group. While the fluorine atom and the OH group are almost identical in size, ATP(γMe) and ATP(γPh) which do not affect the mitochondrial respiration, have larger substituents replacing the OH group in the natural phosphate.

Effects on the mitochondrial ATPase activity

In another series of experiments we have tested the effect of the same phosphate-modified ATP analogs on the 2,4-dinitrophenol-stimulated ATPase activity of intact mitochondria. The results obtained with rat liver mitochondrial ATPase are shown in Table II. Here again, ATP(γF) and AMP-*P*(NH)*P* have quite different effects. Whereas AMP-*P*(NH)*P* is a strong inhibitor of the 2,4-dinitrophenol-stimulated ATPase activity of intact rat liver mitochondria, the fluorophosphate analog ATP(γF), as well as ATP(γMe) and ATP(γPh), do not affect the uncoupler-activated ATP hydrolysis.

On the other hand we have also compared the effect of ATP(γF) and AMP-*P*(NH)*P* on the Mg^{2+}-ATPase activity of submitochondrial 'lubrol particles',

TABLE II

EFFECT OF THE NUCLEOTIDE ANALOGS ATP(γF) AND AMP-*P*(NH)*P* ON THE 2,4-DINITROPHENOL-STIMULATED ATPase ACTIVITY OF INTACT RAT LIVER MITOCHONDRIA

The ATPase medium contained in a final volume of 0.2 ml: 100 mM KCl, 25 mM Tris-HCl (pH 7.4), 0.5 mM EDTA, 2 mM $MgCl_2$, 0.1 mM 2,4-dinitrophenol, 0.7 U pyruvate kinase, 5 mM phospho*enol*pyruvate and 0.06—0.12 mg of rat liver mitochondrial protein. The reaction was initiated with ATP (2 mM final concentration). Incubation time was 10 min at 37°C. The ATPase activity in the absence of an uncoupler was 24 nmol P_i released/min per mg protein. The percent inhibition was calculated by considering the observed ATPase activity of the control experiment as 100%.

Additions	P_i released (nmol/min per mg protein)	% inhibition
Control	342	0
Oligomycin (1.0 μM)	10	97
ATP(γF) (0.5 mM)	344	0
ATP(γ) (1.0 mM)	334	3
ATP(γF) (2.5 mM)	318	7
AMP-*P*(NH)*P* (0.3 mM)	186	46
AMP-*P*(NH)*P* (2.0 mM)	61	82
ATP(γF) (2.5 mM) + AMP-*P*(NH)*P* (0.3 mM)	178	48

which resemble sonicated mitochondria [17,26]. As demonstrated by the traces in Fig. 1B, the Mg^{2+}-ATPase activity of rat liver 'lubrol particles' was strongly inhibited by AMP-*P*(NH)*P*, while a 10—100-fold higher ATP(γF) concentration was practically without effect on the Mg^{2+}-ATPase activity. The

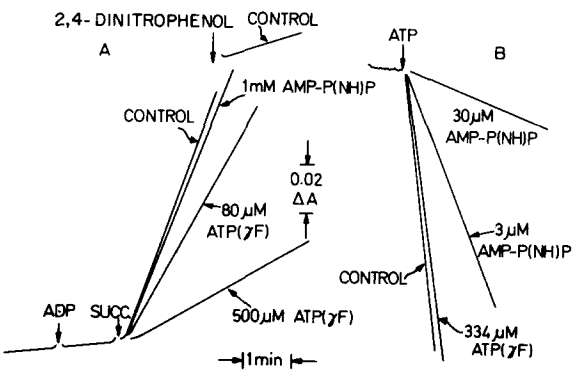

Fig. 1. Effect of ATP(γF) and AMP-*P*(NH)*P* on oxidative phosphorylation (A) and on the Mg^{2+}-ATPase activity (B) of rat liver submitochondrial 'lubrol particles'. (A) The reaction medium contained at a final volume of 1 ml: 250 mM sucrose, 10 mM Tris-HCl (pH 7.4), 0.5 mM EDTA, 5 mM phosphate (pH 7.4), 2.5 mM $MgCl_2$, 2 mg defatted bovine serum albumin, 5 mM glucose, 0.4 mM $NADP^+$, 3 U hexokinase, 1.8 U glucose-6-phosphate dehydrogenase, 0.28 mM AMP and 0.58 mg of protein from 'lubrol particles'. The submitochondrial particles were incubated for 3 min at 24°C prior to initiation of the reaction by addition of 50 μM ADP and 5 mM of succinate. ATP(γF) and AMP-*P*(NH)*P* were present at the concentrations indicated on the traces. The upward deflection of the trace at 366 nm corresponds to a reduction of $NADP^+$. 20 μM of 2,4-dinitrophenol were added to the control sample in order to check whether the $NADP^+$ reduction was linked to the oxidative phosphorylation. (B) The reaction medium contained at a final volume of 1 ml: 100 mM KCl, 20 mM Tris-HCl (pH 7.4), 2 mM $MgCl_2$, 50 μM NADH, 2 U pyruvate kinase, 2 U lactate dehydrogenase, 0.5 mM phospho*enol*pyruvate, 1.5 μM rotenone and 0.08 mg of protein from 'lubrol particles'. The reaction was started by addition of 35 μM ATP. ATP(γF) and AMP-*P*(NH)*P* were present at the concentrations indicated on the traces. The downward deflection of the traces at 366 nm represents the oxidation of NADH.

similar results obtained with intact organelles and with submitochondrial particles eliminates a transport barrier and points to a fundamental difference in the mode of action of ATP(γF) and AMP-*P*(NH)*P* on mitochondrial F_1-ATPases. It has to be pointed out that the inhibition of ATPase by AMP-*P*(NH)*P* is time dependent, in agreement with previous data [7,30].

Therefore, in our experiments we always preincubated the submitochondrial particles for 3 min with AMP-*P*(NH)*P* before initiating the reaction with ATP. Further incubation with the ATP analog did not increase the degree of inhibition. The results obtained with ATP(γF) and AMP-*P*(NH)*P* strongly support the feasibility of selective inhibition of ATP-utilizing and ATP-producing processes.

Effects on oxidative phosphorylation and related reactions

The different effects of ATP(γF) and AMP-*P*(NH)*P* on the ADP-stimulated respiration and on the mitochondrial ATPase activity, prompted us to investigate further the involvement of these analogs in the reactions of mitochondrial oxidative phosphorylation. Their effect on the energy-linked reversal of the electron flow in the respiratory chain is shown in Fig. 2. If ATP was used to promote the succinate-linked NAD^+ reduction in rat liver mitochondria (Fig. 2A), the AMP-*P*(NH)*P* analog inhibited the reversal of the electron flow much more strongly than did ATP(γF). However, when aerobically generated high energy intermediates were used as the energy source for the reduction of intramitochondrial NAD^+ (Fig. 2B), the ATP(γF) analog becomes a very potent inhibitor for the reversal of the electron flow in the respiratory chain, whereas AMP-*P*(NH)*P* does not interfere with this process at all.

The difference between AMP-*P*(NH)*P* and ATP(γF) is also revealed by their effects on the succinate-linked reduction of acetoacetate (Table III). While AMP-*P*(NH)*P*, as well as the natural nucleotide ATP, does not affect this process at all, ATP(γF) acts as an inhibitor of the succinate-linked reduction of

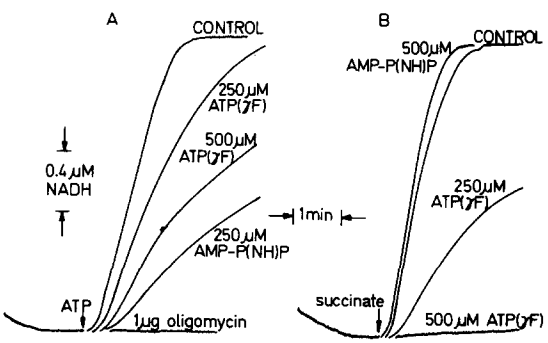

Fig. 2. Effect of ATP(γF) and AMP-*P*(NH)*P* on the reduction of NAD^+ by succinate. (A) With ATP as the source of energy. (B) With aerobically generated high energy intermediates as the source of energy. In both cases the reaction medium contained in a final volume of 1 ml: 250 mM sucrose, 20 mM Tris-HCl (pH 7.4) and 0.85 mg of rat liver mitochondrial protein. Also present were either 0.4 mM Na_2S and 5 mM succinate (A), or 5 mM $MgCl_2$ (B). Additions were ATP(γF), AMP-*P*(NH)*P* or oligomycin at the concentrations indicated on the traces. After 5 min of incubation at 24° C, the reaction was initiated with either 500 μM ATP (A), or with 5 mM succinate (B), as indicated by the corresponding arrows. The fluorescence increase due to NADH formation (the upward deflection of the traces), was measured with an Eppendorf fluorimeter using the appropriate filter combinations (excitation filter 366 nm; emission filter 470—3000 nm).

TABLE III

EFFECT OF ATP(γF) AND AMP-*P*(NH)*P* ON THE SUCCINATE-LINKED REDUCTION OF ACETOACETATE BY RAT LIVER MITOCHONDRIA

The reaction medium contained in a final volume of 0.2 ml: 20 mM glycylglycine (pH 7.5), 50 mM KCl, 130 mM sucrose, 0.5 mM EDTA, 10 mM $MgCl_2$, 5 mM lithium acetoacetate, and 1.2 mg of rat liver mitochondrial protein. The reaction, at 24°C, was started with 12.5 mM succinate. Nucleotides were present at the indicated concentrations. After 20 min of incubation, the reaction was stopped with perchloric acid and the remaining acetoacetate measured enzymatically in the KOH-neutralized extract using an excess of NADH and 3-hydroxybutyrate dehydrogenase. The succinate-independent reduction of acetoacetate was subtracted for each sample before calculating the percent inhibition due to individual nucleotides. The results are the mean value from usually three, but at least two separate experiments.

Additions	nmol acetoacetate reduced/ min per mg protein	% inhibition
No succinate	1.7	—
Control	16.3	—
Antimycin (0.3 μg)	3.9	85
ATP (1.5 mM)	15.6	5
ATP(γF) (0.3 mM)	10.2	42
ATP(γF) (1.2 mM)	6.0	71
AMP-*P*(NH)*P* (0.5 mM)	15.8	3
AMP-*P*(NH)*P* (1.5 mM)	16.8	0

acetoacetate catalysed by rat liver mitochondrial preparations.

When we tested the effect of GTP(γF) on mitochondrial respiration, this fluorophosphate-containing guanine nucleotide analog showed no inhibitory effect on the reactions of oxidative phosphorylation, indicating that the specificity of interaction of ATP(γF) with mitochondrial membrane systems requires both the fluorophosphate group and an intact adenine moiety.

In order to evaluate possible differences in the interaction of ATP(γF) and AMP-*P*(NH)*P* with inner mitochondrial membrane components, further studies were carried out on submitochondrial particles with no membrane barrier between the enzymes and the test medium containing the ATP analogs. As demonstrated by the traces in Fig. 1A, ATP(γF) inhibits the oxidative phosphorylation of 'lubrol particles', whereas AMP-*P*(NH)*P* (as well as ATP(γMe) and ATP(γPh), not included in this graph), is ineffective even at concentrations as high as 1 mM. At high ATP(γF) concentrations (greater than 1 mM), a slight uncoupling effect could be observed in the 'lubrol' particles', the *P/O* ratio decreased from 0.40—0.45 to 0.32—0.37. However, at low nucleotide concentrations, 4—20 μM ADP or 10—50 μM ATP(γF), we found a competition between ADP (K_m = 4.5 μM) and ATP(γF) (K_i = 17 μM) for the same site involved in the ATP synthesis. Since in these experiments we did not find a time-dependent inhibition of oxidative phosphorylation as with intact mitochondria, it seems that in the latter case the permeability barrier plays an important role in the inhibition by ATP(γF).

The effect of ATP(γF) on the succinate-linked reduction of NAD^+ via aerobically generated high energy intermediates is unusual for this class of compounds. It is interesting that so far none of the investigated natural or modified nucleotides have been shown to inhibit this reaction, except uncouplers or inhibitors of the respiratory chain acting on the NADH-coenzyme Q or

succinate-coenzyme Q complexes. This phenomenon requires further investigation.

Concluding remarks

Of the various inhibitors of energy-utilizing reactions in mitochondria, ATP analogs are potentially the most specific, due to the particular role of adenine nucleotides in mitochondrial functions. Therefore, it seems of considerable interest that the phosphate-modified ATP analogs ATP(γF) and AMP-*P*(NH)*P* have complementary effects with regard to ATP-yielding and ATP-driven reactions in mitochondria. This complementarity could mean that the F_1 locus contains two catalytic sites, as indeed suggested by Penefsky [7], one specialized for ATP synthesis which would be more sensitive to ATP(γF), and another specialized in ATP-utilizing reactions, more sensitive to AMP-*P*(NH)*P*. However, an alternate possibility must be considered, namely that the ATP(γF) analog does not interact with the catalytic site, but rather binds to a regulatory site of F_1 [9,31] and therefore is affecting only the rate-limiting step in ATP synthesis.

The different sensitives of mitochondrial preparations of various origins to ATP(γF), suggests the existence of a possible relationship between the lipid content and their inhibitory activity. This indicates that nucleotide analogs such as ATP(γF) could also engage in unspecific interactions with mitochondrial membranes or membrane components.

It is hoped that the use of more ATP analogs, some of which might have complementary effects, such as ATP(γF) and AMP-*P*(NH)*P*, will permit a more specific approach to the study of ATP-producing and ATP-dissipating reactions in mitochondria.

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References

1 Yount, R.G. (1975) in Advances in Enzymology and Related Areas of Molecular Biology (Meister, A., ed.), Vol. 43, pp. 1—56, Wiles and Sons, New York
2 Robins, R.K. (1975) Ann. N.Y. Acad. Sci. 255, 597—610
3 Schlimme, E., Boos, K.S., Bojanovski, P. and Lüstorff, J. (1978) Angew. Chem., Int. Edn. Engl. 16, 695—702
4 Duée, E.D. and Vignais, P.V. (1968) Biochem. Biophys. Res. Commun. 30, 546—553
5 Vignais, P.V., Duée, E.D., Colomb, M., Reboul, A., Cheruy, A., Bârzu, O. and Vignais, P.M. (1970) Bull. Soc. Chim. Biol. 52, 471—497
6 Klingenberg, M., Grebe, K. and Scherer, B. (1971) FEBS Lett. 16, 253—256
7 Penefsky, H.S. (1974) J. Biol. Chem. 249, 3579—3585
8 Philo, R.D. and Selwyn, M.J. (1974) Biochem. J. 143, 745—749
9 Schuster, S.M., Ebel, R.E. and Lardy, H.A. (1975) J. Biol. Chem. 250, 7848—7853
10 Schlimme, E., Lamprecht, W., Eckstein, F. and Goody, R.W. (1973) Eur. J. Biochem. 40, 485—491

11 Eckstein, F. (1975) Angew. Chem., Int. Edn. Engl. 14, 160—166
12 Melnick, R.L. and Donohue, T. (1976) Arch. Biochem. Biophys. 173, 231—236
13 Penefsky, H.S., Garrett, N.E. and Chang, Ta-min (1976) in The Structural Basis of Membrane Function (Hatefi, Y. and Djavadi-Ohaniance, L., eds.), pp. 69—79, Academic Press, New York
14 Kaplan, R.S. and Coleman, P.S. (1978) Biochim. Biophys. Acta 501, 269—274
15 Kezdi, M., Mantsch, H.H., Muresan, L., Tărmure, C. and Bârzu, O. (1973) FEBS Lett. 33, 33—36
16 Jebeleanu, G., Ty, N.G., Mantsch, H.H., Bârzu, O., Niac, G. and Abrudan, I. (1974) Proc. Natl. Acad. Sci. U.S. 71, 4630—4634
17 Bârzu, O., Kiss, L., Bojan, O., Niac, G. and Mantsch, H.H. (1976) Biochem. Biophys. Res. Commun. 73, 894—902
18 Mantsch, H.H., Goia, I., Kezdi, M., Bârzu, O., Dânsoreanu, M., Jebeleanu, G. and Ty, N.G. (1975) Biochemistry 14, 5593—5601
19 Bârzu, O., Abrudan, I., Proinov, I., Kiss, L., Ty, N.G., Jebeleanu, G., Goia, I., Kezdi, M. and Mantsch, H.H. (1976) Biochim. Biophys. Acta 452, 406—412
20 Haley, B. and Yount, R.G. (1972) Biochemistry 11, 2863—2871
21 Eckstein, F., Bruns, W. and Parmeggiani, A. (1975) Biochemistry 14, 5225—5232
22 Randerath, K. and Randerath, E. (1967) Methods Enzymol. 12A, 323—347
23 Benga, G., Muresan, L., Hodârnău, A. and Dancea, S. (1972) Biochem. Med. 6, 508—521
24 Hodârnău, A., Dancea, S. and Bârzu, O. (1973) J. Cell Biol. 59, 222—227
25 Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
26 Chan, T.L., Greenawalt, J.W. and Pedersen, P.L. (1970) J. Cell Biol. 45, 291—305
27 Ernster, L. and Lee, C.P. (1967) Methods Enzymol. 10, 729—738
28 Bertina, R.M., Steenstra, J.A. and Slater, E.C. (1974) Biochim. Biophys. Acta 368, 279—297
29 Papa, S., Tuena de Gómez-Poyou, M. and Gómez-Poyou, A. (1975) Eur. J. Biochem. 55, 1—8
30 Harris, D.A. (1978) Biochem. Soc. Trans. 5, 1278—1281
31 Schuster, S.M., Ebel, R.E. and Lardy, H.A. (1975) Arch. Biochem. Biophys. 171, 656—661