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On the Availability of Certain Metabolites at the Outer Surface of Normal and Malignant Cells for the Membranous de Novo Synthesis of ATP and Other Nucleotides

G. ÅGREN and G. RONQUIST

From the Institute of Medical and Physiological Chemistry, Biomedical Centre, Uppsala, Sweden

ABSTRACT

The formation of ATP at the cell surface of intact glia and glioma cells in culture has been established. The ATP-forming capacity at the surface of the malignant cells was several times greater than that of the normal glia cells. The ATP-forming capacity was about the same on reincubation one hour after the first incubation. The cells were kept in Eagle's medium in the meantime. ADP, NAD^+ and 3-phosphoglyceraldehyde could all be available from a postulated intramembranous metabolic pool and take part in biochemical reactions at the cell surface, provided that albumin was not present in the incubation medium. An incubation medium which was complete except for 3-phosphoglyceraldehyde was only slightly less effective as regards ATP formation at the surface of both glia and glioma cells, compared with the complete incubation medium. The presence of nucleoside diphosphate kinase at the glioma cell surface was confirmed. When intact cells were incubated with only the phosphoryl group donor (ATP) of the reaction but with the acceptor nucleoside diphosphates (CDP, GDP, UDP) omitted, only CTP and GTP were formed. No UTP was found. These latter results indicate that both CDP and GDP are available from the postulated intramembranous metabolic pool, while UDP is not.

INTRODUCTION

Evidence is accumulating for the existence of an intra-membranous metabolic pool in the plasma membrane of animal cells (3, 10, 16). Some of the membrane-associated biochemical reactions have been studied in intact cells. Such studies have the advantage of offering only one side of the plasma membrane to take part in the reaction. A prerequisite condition for the study of the cellular outside is that the substrates and cofactors involved in the enzymatic reaction do not penetrate the membrane in any direction. It is of course necessary that the chemical structure of the membrane and the cellular integrity are not altered during the preparatory steps and cell incubation. Therefore, the cells must be treated gently and always suspended in physiological buffers during a limited period of time. Furthermore, we have found (1, 2, 3, 4), in agreement with others (9, 20), that albumin and, under certain conditions, Dextran T40 have been most useful as "stabilizers" for the plasma membrane. Taking all these reservations into consideration, we conclude that several important biochemical reactions can occur at the cell surface, e.g. the *de novo* synthesis of ATP and other nucleotides. These reactions required the presence of all necessary substrates and cofactors. If one (or several) of them was excluded, the reaction scarcely occurred at all (5). However, although the endogenous metabolites were not always available for reactions at the cell surface, we still had reason to believe that the metabolites were present intramembranously. One basis for such an assumption was our early observations that pure human erythrocyte membranes could form both ATP and important intermediates of the phospholipid biosynthesis without the addition of any substrate to membranes, except (^{32}P)orthophosphate (13, 14).

The hypothesis that the availability of intramembranous metabolites at the outer surface of the plasma membrane might depend upon the presence of albumin or Dextran T40 in the external medium was therefore tested. The present paper deals with results from experiments where normal and malignant cells have been examined as regards the capacity of *de novo* synthesis of ATP and other nucleoside triphosphates at the cell surface in the absence of albumin and Dextran.

MATERIAL AND METHODS

The description of the cell lines and of the preparation of Ehrlich cells and glia and glioma cells has been given in previous papers (3, 6). In the experiments where neither albumin nor Dextran was present in the incubation media, these polymers were also excluded from the washing media during preparation.

The glia and glioma cells were incubated directly in the

Table I. *ATP formation at the membrane surface of human glia and glioma cells in culture*

	Cells incubated once ATP formed	Cells incubated twice ATP formed
251 MG (malignant cells)	15.4	3.8
105 MG (malignant cells)	7.2	9.2
138 MG (malignant cells)	4.3	3.0
403 CG (normal cells)	0.95	6.7

The values denote the (^{32}P)orthophosphate incorporation into ATP in pmol/1×10^6 cells/min at 37°C (mean values of two experiments). Cells incubated once are cells incubated in the usual way. Cells incubated twice are cells which were first incubated once and then washed and resuspended in Eagle's medium for 1 hour. These cells were then again incubated in the usual way. The values given represent the results from the second incubation only.

Petri dishes while still attached to the dishes. The technique has been described in previous papers (2, 3). To the complete incubation medium containing 130 mM NaCl and 25 mM KCl to maintain isotonicity with or without 1.5% Dextran T40 for ATP-synthesis at the membrane surface, the following substances were added: 150 μmoles of Tris acetic acid buffer, pH 7.5; 15 μmoles of $MgCl_2$; 3 μmoles of glutathione (reduced form); 3 μmoles of (^{32}P)orthophosphate; 4.5 μmoles 3-phosphoglyceraldehyde; 6 μmoles of NAD^+ and 7 μmoles of ADP. Total incubation volume was 3 ml. This incubation medium is called the *complete medium*. The termination of reaction and pooling of incubation media were performed in accordance with a previous paper (2). The isolation of radioactive nucleotides and the radiometric and chemical determination was in accordance with a previous work (11).

The presence of nucleoside diphosphate kinase at the intact cell surface of glioma cells has been demonstrated. (3). These experiments were all performed in the presence of a terminally labelled nucleoside triphosphate as a phosphoryl donor and a nucleoside diphosphate as a phosphoryl acceptor. The technique in the present report is identical with that reported in (3), except that the acceptor nucleoside diphosphate was always omitted from the medium.

RESULTS

Three different malignant cell lines are all capable of forming ATP at the cell surface in an amount which is severalfold higher than that of the normal glia cells (Table I). Thus, cells still attached to the culture plates can form extracellular ATP in the same way as do cells which have been scraped off the plates and suspended in the isotonic medium (1).

Some enzymes, and probably other proteins of the surface membrane, can be eluted into the suspending medium (3, 12, 15, 19). Certain metabolites have proved very efficient as eluting agents (17, 19). Therefore, it was interesting to see how the ATP-forming capacity was at the membrane surface during an additional incubation after an intermediate period of 1 hour in Eagle's medium of cells already incubated once.

As presented in Table I, two of the malignant cell lines showed a decreased activity on reincubation, while the third one (105 MG) showed a slightly increased activity. The normal glia cells showed an increased activity on reincubation.

Table II illustrates the effect of separately omitting each one of the three substrates and cofactors from the complete incubation medium of glioma cells when omitting albumin and Dextran T40 from the incubation medium. As is seen in the table, the most significant decrease in ATP formation is achieved in the absence of NAD^+ in the incubation medium. An intermediate effect is obtained by the omission of ADP, while the omission of 3-phosphoglyceraldehyde results in only a rather small decrease. The same procedure has been carried out also with normal glia cells (Table 3). The general tendency was rather similar, even though the glia cells seemed to be less dependent on the presence of NAD^+.

A series of experiments was also carried out in order to determine the availability of different nucleoside diphosphates for the nucleoside diphosphate kinase reaction, with ATP as the only nucleotide added to the incubation medium. The isolation procedure for the different nucleoside-triphosphates (CTP, GTP, UTP) has been basically the same as was described in (3), but it was necessary to introduce some important modifications. The stages of the elution in the ion exchange

Table II. *ATP formation at the membrane surface of human glioma (251 MG) cells in culture in either a complete medium or a complete medium lacking one cofactor or substrate*

	Glioma cells
Complete medium	100
Complete medium minus 3-phospho-glyceraldehyde	79.7
Complete medium minus ADP	38.6
Complete medium minus NAD^+	19.9

The figures given are percentage values of the ATP-forming capability in the surface membrane under the different incubation conditions where the ATP formation under optimal conditions (complete medium) has been given 100%.

Table III. *ATP formation at the membrane surface of human glia cells (622 CG) in culture in either a complete medium or a complete medium lacking one cofactor or substrate*

	Glia cells
Complete medium	100
Complete medium minus 3-phospho-glyceraldehyde	75.2
Complete medium minus ADP	43.9
Complete medium minus NAD^+	41.8

The figures given are percentage values of the ATP-forming capability in the surface membrane under the different incubation conditions where the ATP formation under optimal conditions (complete medium) has been given 100%.

chromatography were the following: 4 N formic acid, 4 N formic acid+0.2 M ammonium formate, 4 N formic acid+0.3 M ammonium formate, 4 N formic acid+0.4 M ammonium formate, 4 N formic acid+0.7 M ammonium formate, 4 N formic acid+1 M ammonium formate. In addition, to obtain good separation, the various nucleotides had to be rechromatographed in the same system either once (GTP, UTP) or twice (CTP). A consistent finding was that GTP and CTP were readily formed in the absence of the corresponding nucleoside diphosphates but with ATP in the incubation media. By contrast, UTP was shown never to occur in the absence of UDP. Thus, it is evident that GDP and CDP are more readily available at the surface of the membrane, while UDP is not.

DISCUSSION

The results presented show that extracellular ATP can also be formed by cells attached to the plates throughout the experiment. This is in agreement with a previous work where cells were scraped off the plates and maintained free in an isotonic solution during the experiment (1). Likewise, the ATP-forming capacity was higher for the malignant glioma cells than for the normal glia cells, when compared with three different malignant lines.

Practically no difference in activity was observed on reincubation of two of the malignant cell lines. This means that the first incubation procedure and successive incubation in Eagle's medium did not remove the ATP-forming activity at the cell surface by total elution. This finding strengthens the view that this activity is due not to adsorbed enzyme at the cell surface but is an integral part of the plasma membrane with some of its activity facing the outer cell surface (3). One of the malignant cell lines showed a decrease in activity on reincubation, while the normal glia cells were more active. Because the pattern is not uniform for the different cell lines, the increased activity of the normal glia cells is probably not explained by simple leakage of intracellular enzymes. The increase in activity of normal cells on reincubation up to the level of malignant cells is worthy of interest. The experimental conditions of the preceding procedure may have brought about a change in the architecture of the surface membrane of the normal cells. Such a change would be analogous to reports of transformations produced by viral and chemical agents and also proteases (7, 8). In the present experiments the protease treatment might be considered to have been replaced by an elution of some proteins at the cell surface (11, 17, 19).

We reported in an earlier paper (5) that the ATP formation at the cell surface of Ehrlich cells could only occur in the presence of all substrates and cofactors. These results were obtained with albumin or Dextran added to the incubation media as membrane "stabilizers" (9, 20). The present results on glia and glioma cells were all obtained in the absence of albumin or Dextran from the media. Under these conditions there is only a slight decrease in the ATP-forming capacity at the cell surface when 3-phosphoglyceraldehyde is lacking in the otherwise complete incubation medium. This decrease is more pronounced when ADP, rather than 3-phosphoglyceraldehyde, is lacking. This observation indicates a high degree of specificity in the membrane architecture with regard to the localization of the different metabolites.

The reaction catalysed by nucleoside diphosphate kinase at the cell surface supports the same conclusion. Here again, a high degree of specificity was seen. Thus, both GTP and CTP were readily formed at the cell surface in an incubation medium lacking GDP, CDP and UDP but with ATP present. Under these conditions, no UTP could be isolated. Therefore the GTP and CTP formation was probably not due to simple leakage of the corresponding nucleoside diphosphate from the cell interior.

These results strongly support our view that an intramembranous metabolic pool exists. Some of its metabolites may be available in differing degrees at the cell surface, at low concentrations (less than 0.1%) of albumin, or in the absence of it. This in-

dicates a possible important role of albumin *in situ*, provided that the albumin concentration in the interstitial fluid in the brain parenchyma is as low as 0.1 % or lower (19). If such is the case, ATP synthesis might occur at the glia and glioma cell surface *in vivo* since ADP, NAD^+ and 3-phosphoglyceraldehyde are available at the cell surface under this condition. This ATP might have important functions at the cell membrane, e.g. regulatory phosphorylation between cells (15).

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Address for reprints:

Gunnar Ågren, M.D.
Institute of Medical and Physiological Chemistry
Box 575
S-751 23 Uppsala
Sweden