Somatic Cell Genetics, Vol. 8, No. 6, 1982, pp. 723–730

Enhancement of Polyethylene Glycol-Mediated Cell Hybridization by Inducers of Erythroleukemia Cell Differentiation

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Received 30 March 1982—Final 14 June 1982

Abstract—*Dimethyl sulfoxide (DMSO) has many biological effects, which include enhancement of polyethylene glycol (PEG) -mediated cell fusion, induction of cell differentiation in erythroleukemia and other cell systems, and cryoprotection of cells from freezing damage. In this study, compounds which induce erythroleukemia cell differentiation were tested for their ability to enhance PEG-mediated cell fusion. It was found that many compounds which induce erythroleukemia cell differentiation also promote cell membrane fusion as well as protect cells against freezing damage. Hence, many inducers of erythroleukemia cell differentiation have direct and similar effects on cell membranes. This study also demonstrates previously unrecognized effects of cryoprotective agents and cell fusogens on the differentiated state of cultured cells.*

INTRODUCTION

Cultured erythroleukemia cells can be induced to undergo erythroid differentiation by treatment with a number of structurally unrelated compounds (1). Inductive agents include dimethyl sulfoxide (DMSO) (2), triethylene glycol (3), hemin (4), certain fatty acids (5, 6), purine and nicotinamide derivatives (7), ouabain (8), and a number of polar organic compounds (1, 3, 9). DMSO, the widely employed erythroleukemia cell differentiation inducer, is a well-known cryoprotective agent (10, 11) and is also effective in enhancing the cell fusion properties of polyethylene glycol (PEG) (12). Since DMSO acts synergistically with PEG in inducing cell membrane fusion, other inducers of erythroleukemia cell differentiation have been surveyed in this

0098-0366/82/1100-0723$03.00/0

study for their ability to promote PEG-induced cell fusion. It has been found that many inducers of erythroleukemia cell differentiation promote cell fusion. While the results presented do not rule out a direct action of inductive agents on chromosomal nucleoproteins, clear evidence is provided which indicates that many inducers of erythroleukemia cell differentiation have both direct and similar effects on cell membranes.

MATERIALS AND METHODS

Maintenance of Cells. Mouse LM(TK$^-$) cells (13) and mouse RAG renal adenocarcinoma cells, which lack hypoxanthine phosphoribosyltransferase (14), were maintained in Dulbecco's medium supplemented with 1 mM proline, 10% newborn calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. For the selection of hybrid cells, HAT medium (15, 16) was prepared by the proper supplementation of Dulbecco's medium.

Assay for Compounds Which Enhance Cell Hybridization Properties of PEG. Several erythroleukemia differentiation inducers, cryoprotective agents, and chaotrophic agents were surveyed for their ability to enhance the cell hybridization properties of PEG. In these studies, a mixed cell population, bearing appropriate genetic markers for the isolation of somatic cell hybrids via the HAT selection system (15, 16), was treated with 25% PEG-600 plus a test compound. Cell populations treated with 25% PEG-600 alone produced few somatic cell hybrids while treatment with 25% PEG-600 plus compounds similar to DMSO generated large numbers of hybrids. PEG alone produces optimum numbers of hybrid cells at a concentration of approximately 50% (17, 18). Technical details of the cell fusion procedures employed have been described previously (18). In brief, 2.5×10^5 LM(TK$^-$) cells and 2.5×10^5 RAG cells were planted in 25-cm^2 tissue culture flasks (Corning Glass Works, Corning, New York). After 15 h, the cell monolayer was washed with minimal attachment medium lacking Ca^{2+} (MAM-Ca^{2+}) and then treated with a cell fusion solution. Solutions containing cell fusogens were prepared with 0.15 M HEPES, pH 7.55, and sterilized by membrane filtration. We have previously found that increased numbers of hybrids are recovered when cells are fused with fusogens dissolved in 0.15 M HEPES, pH 7.55, rather than serum-free culture medium (18). Following a 1-min exposure to a fusogen, the cell monolayer was washed three times with MAM-Ca^{2+} for 10 sec/wash, incubated for 30 min in MAM-Ca^{2+}, and then treated with HAT medium in order to select for somatic cell hybrids. After 7 days, the hybrids induced were stained and enumerated.

Reagents. The sources of compounds tested for their ability to enhance the hybridization properties of PEG-600 are given in Table 1. All other compounds were of reagent grade.

RESULTS

A sensitive assay for compounds which enhance PEG-mediated cell hybridization was devised. The principle of the assay employed is based on two observations. First, cell hybridization mediated by PEG occurs within a narrow range of concentrations (17, 18). For example, while 50% PEG produces optimal numbers of hybrid cells, PEG at concentrations below 45% or above 60% yields few hybrid colonies (17). Second, Norwood et al. (12) found that 10–15% DMSO enhanced the frequency of multinucleated cells treated with suboptimal concentrations of PEG. It was determined that 10% DMSO produced a maximum increase in hybrid colonies when PEG-600 was employed at concentration of 25%. Thus, in screening for new compounds which enhance PEG-mediated cell hybridization, 25% PEG-600 was employed.

Since DMSO has well-known effects as a cryoprotective agent (19–21) and as an inducer of erythroleukemia cell differentiation (1, 2), other compounds with similar biological properties were screened for their ability to enhance PEG-mediated cell hybridization (Table 1). Twenty-two compounds were detected which acted synergistically with 25% PEG-600. Since compounds which acted synergistically with 25% PEG-600 also behaved synergistically with 30% PEG-600 (Table 1), synergistic activity occurred over a range of concentrations. The relative effectiveness of each compound was calculated from the following ratio:

$$\text{Fold enhancement of cell hybridization} = \frac{\text{hybrid colonies elicited by 25\% PEG-600 + test compound}}{\text{hybrid colonies elicited by 25\% PEG-600 alone}}$$

Compounds which enhanced PEG-mediated cell hybridization are listed in Table 1 in decreasing order of effectiveness. Ten compounds were detected which were more effective than DMSO in enhancing PEG-mediated cell hybridization. PEG-200, which is a poor promoter of cell hybridization (17), was among the most effective enhancers of PEG-600-mediated cell hybridization and ethylene glycol was moderately effective (Table 1). While DMSO is well known as an inducer of erythroleukemia cell differentiation (2), a number of compounds have been detected which are considerably more effective in inducing erythroleukemia cell differentiation (1). Many agents which are more effective than DMSO in inducing erythroleukemia cell differentiation are also more effective than DMSO in enhancing PEG-mediated cell hybridization (Table 1, see acetamide, pyridine-*N*-oxide, PEG-200, *N*-methylacetamide, *N*, *N*'-dimethylacetamide, and *N*, *N*'-dimethylformamide). It is interesting to note that low-molecular-weight PEG (triethylene glycol) is

Table 1. Synergistic Action of Various Compounds with PEG in Promotion of Cell Hybridization[a]

	Number of experiments	-Fold enhancement of cell hybridization	Optimum concentration (molar)	Optimum concentration for induction of erythroleukemia cell differentiation (molar)	Optimum concentration for cryoprotective effect
Acetamide (1)[b]	3*	15.7	1.0	0.25	0.1
Potassium Bromide (2)	2*	15.1	0.03		0.1
Urea (2)[c]	2*	15.0	0.25		0.3
Glycerol (3)	2*	15.0	2.1		0.55
Pyridine-N-oxide (1)	2*	12.0	1.0	0.1	0.01
PEG-200 (4)	2*	11.0	1.0	0.25	1.0
N-Methylacetamide (2)	2*	10.5	1.5	0.05	1.0
N,N′-Dimethylacetamide (1)	1*	9.0	1.0	0.03	0.1
N,N′-Dimethylformamide (2)	2*	7.7	1.36	0.15	0.1
Sodium Sulfate (4)	3*	6.0	0.1		0.001
DMSO (4)	>60*	5.1	2.5	0.28	0.3
Sodium Acetate (4)	2*	4.3	0.04	0.06	—
N-Methylformamide (1)	2*	4.0	1.2	0.25	0.1
Glucose (4)	2*	3.1	0.25		1.0
Sodium Propionate (2)	1*	3.0	1.2	0.0025	—
Methyl Sulfone (1)	1*	2.8	2.0		0.1
Sucrose (4)	1	2.8	0.25		1.0

Tetramethylene Sulfone (1)	3*	2.4	0.1		0.01
Hydroxyethyl Starch (4)	1	2.0	0.65%		1%
Sodium Butyrate (4)	2*	2.0	0.1	0.001	—
Ethylene Glycol (1)	2*	1.6	0.25		3.2
Valerolactam (1)	2*	1.6	0.3	0.1	0.001

[a]The results of a survey of 22 compounds for synergistic action with 25% PEG-600 in the promotion of cell hybridization is presented. The number of tests for synergism with 25% PEG-600 is given in the table (an asterisk indicates that synergism was noted following one or more experiments employing 30% PEG-600 as well). Compounds were surveyed over a wide range of concentrations for synergistic action with PEG. In each experiment, two or more concentrations of a synergistic agent generally resulted in enhanced cell hybridization. The optimum synergistic concentration determined is given above. All concentrations given in the table are molar unless otherwise indicated. The -fold enhancement of cell hybridization was calculated from the ratio of hybrid colonies produced by 25% PEG-600 plus a test compound to hybrid colonies produced by 25% PEG-600 alone. The -fold enhancement of cell hybridization promoted by the optimum synergistic concentration is presented and the compounds studied are listed in decreasing order of their synergistic effect. Each experiment was carried out with three controls: (1) 25% PEG-600 without a synergistic compound, (2) 25% PEG-600 + 10% DMSO, and (3) 50% PEG-600. While the optimal PEG-600 concentration (50%) produced an average of 100.2 hybrids/flask, the suboptimal 25% PEG-600 concentration yielded an average of 5.3 hybrids/flask. Evidence is drawn from the literature concerning the effectiveness of the compounds studied as inducers of erythroleukemia cell differentiation and as cryprotective agents. If an entry is not made in the table, no data were found in the literature for the activity in question. See text references 1, 3, and 6 for the sources of optimum concentrations for various inducers of erythroleukemia cell differentation and text reference 21 for the optimum concentration for cryoprotective agents.

[b]Sources of chemicals are indicated by numbers in parentheses: (1) = Aldrich Chemical Co., Milwaukee, Wisconsin; (2) = J.T. Baker Chemical Co., Phillipsburg, New Jersey; (3) Fisher Scientific, Houston, Texas; (4) = Sigma Chemical Co., St. Louis, Missouri.

[c]Cyanates were removed from urea by passage through a Dowex ion-exchange column.

itself an inducer of erythroleukemia cell differentiation (1). It is noteworthy that selenium oxide, which is the most potent known inducer of erythroleukemia cell differentiation (22), was ineffective in enhancing PEG-mediated cell hybridization. This observation may indicate that mechanistic differences exist between selenium oxide and the organic compounds listed in Table 1.

For all but one compound studied, higher concentrations were required to enhance PEG-mediated cell fusion than to promote erythroid differentiation (Table 1). Differences in the concentrations required for optimal cell fusion versus erythroid differentiation may be a result of differences in the duration of the treatments employed. For the promotion of cell fusion, cells are exposed to a fusogen for only 1 min, while for induction of erythroid differentiation, continuous treatment with an inductive agent for a period of days is required.

Since DMSO is commonly employed as a cryoprotective agent (11, 19, 20), several compounds with cryoprotective activity were tested for their ability to enhance PEG-mediated cell hybridization (Table 1). Nineteen cryoprotective agents acted synergistically with PEG in promoting cell hybridization (Table 1). It is noteworthy that cell survival following cryoprotection with PEG-3000 or ethylene glycol is superior to the cryoprotective effect elicited by DMSO (21).

DISCUSSION

This study demonstrates the existence of a class of compounds which share several apparently unrelated biological properties. While DMSO is a well-recognized cryoprotective agent (19, 20), inducer of erythroleukemia cell differentiation (1, 2), and enhancer of cell hybridization (12), many compounds which share all of the above biological properties have been detected (Table 1). Members of this class of compounds have also been found to be active inducers of interferon production (23). Ten compounds were found to enhance PEG-mediated cell hybridization more effectively than DMSO itself (Table 1), and a number of compounds have previously been described which are more effective than DMSO as cryoprotective agents (21) and inducers of erythroleukemia cell differentiation (1, 3). Thus, DMSO is far from unique and is, in fact, less effective than many other compounds.

The evidence presented indicates that many structurally unrelated inducers of erythroleukemia cell differentiation share the ability to act synergistically with PEG in promoting cell fusion. Thus, inducers of erythroleukemia cell differentiation have specific effects on cell membranes. DMSO, in particular, has been associated with a variety of membrane effects, e.g., reduction in membrane permeability to phosphate, uridine, and leucine (24); alteration in the microviscosity of membranes (25, 26); increase in lectin agglutinability of cell-surface molecules (26, 27); increase in the melting

temperature of acyl chains in artificial phospholipid membranes (28); and cryoprotection of cell membranes (2). The present study clearly demonstrates that many inducers of erythroleukemia cell differentiation and cryoprotective agents can promote at least one specific membrane perturbation, i.e, cell membrane fusion. The apparently unrelated biological effects of DMSO-like compounds may be due to a common cell membrane alteration with pleotrophic effects.

In addition to demonstrating the membrane active properties of many inducers of erythroleukemia cell differentiation, this study also had two other unexpected consequences. First, compounds used to promote cell fusion and to protect cells against freezing damage may have previously unrecognized effects on the differentiated state of cultured cells. Secondly, since cell fusion can result in the liberation of latent viruses (29) and the alteration of the differentiated state of cells (30), the compounds identified in this study may represent a biohazard to man.

ACKNOWLEDGMENTS

We wish to thank Ms. Sharon Labardini and Ms. Judy Kelley for their aid in preparation of the manuscript. This study was supported, in part, by grants GM 31056 and CA 33074.

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