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MEMBRANE MOBILITY AGENTS

A NEW CLASS OF BIOLOGICALLY ACTIVE MOLECULES

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SUMMARY

A number of cyclopropane fatty acid esters which promote the lateral mobility of fluorescent antibody-labeled antigenic sites through lymphocyte membranes (measured by rate and extent of cap formation) are described. The effect is dependent on molecular structure and dose; the compounds are termed membrane mobility agents.

Molecules ranging in size from phospholipids to surface antigens move laterally through cell membranes, facts conveniently represented by the fluid mosaic model formulated by Singer and Nicolson [1]. Molecular mobility rises with increasing membrane fluidity (local [2–4] or general) which, in turn, is related to the proportion of unsaturated alkanoic acid residues present in both natural [5] and bilayer model [6] membranes. Scattered evidence shows that assembly of membrane transport systems [7] and the membrane functions of transport [8–10] and permeability [11] are augmented with increased membrane fluidity [12]. Agents which increase membrane fluidity might produce useful and interesting biological changes. We have therefore synthesized a series of compounds which promote the motion of fluorescent antibody-labeled antigenic sites on lymphocytes, and thus may be classified as membrane mobility agents.

Molecules of membrane mobility agents consist of two parts, a hydrophobic chain with disorder-promoting substituents, and a hydrophilic chain which bridges the gap between the hydrocarbon and outer regions of the membrane. The fluidity-promoting hydrophobic chain in natural membranes is an unsaturated fatty acid, but these are subject to autooxidation. Conversion of double bonds to cyclopropane rings by the Simmons–Smith reaction [13] removes the O_2 sensitivity [14] and preserves approximately the disorder-promoting geometry of the double bond. Interestingly, the unsaturated fatty acids present in the membranes of rapidly growing

TABLE I

MEMBRANE MOBILITY AGENTS

All compounds were carefully purified by chromatography. Cyclopropane compounds contained no detectable carbon–carbon double bonds (NMR). Criteria for purity were: thin-layer chromatography (single spot), gas–liquid chromatography (>97 % pure), infra red and NMR spectra in agreement with structures.

Abbreviation	Formula	Systematic name
A_1C	$CH_3(CH_2)_7$-1-$(CH_2)_7COOCH_2CH_2OCH_3$	2-methoxyethyl 8-(2-*n*-octylcyclopropyl)-octanoate 2-methoxyethyl 9,10-methyleneoctadecanoate 2-methoxyethyl-dihydrosterculate
A_2C	$CH_3(CH_2)_7$-1-$(CH_2)_7COOCH_2CH_2OCH_2CH_2OCH_3$	2-(2-methoxy)-ethoxyethyl 8-(2-*n*-octylcyclopropyl)-octanoate 2-(2-methoxy)-ethoxyethyl 9,10-metholeneoctadecanoate
A_3C	$CH_3(CH_2)_7$-1-$(CH_2)_2COOCH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$	2-(2-methoxy)-ethoxyethyl-dihydrosterculate 2-[2-(2-methoxy)-ethoxy]ethoxyethyl 8-(2-*n*-octylcyclopropyl)-octanoate 2-[2-(2-methoxy)-ethoxy]ethoxyethyl 9,10-methylene-octadecanoate 2-[2-(2-methoxy)-ethoxy]ethoxyethyl-dihydrosterculate
A_2C_2	$CH_3(CH_2)_4$-1-CH_2-1-$(CH_2)_7COOCH_2CH_2OCH_2CH_2OCH_3$	2-(2-methoxy)-ethoxyethyl-8-[2-(2-*n*-pentylcyclopropyl-methyl)-cyclopropyl]-octanoate 2-(2-methoxy)-ethoxyethyl-*bis*-9,10,12,13-methylene-octadecanoate
A_2C_3	CH_3CH_2-1-CH_2-1-CH_2-1-$(CH_2)_7COOCH_2CH_2OCH_2CH_2OCH_3$	2-(2-methoxy)-ethoxyethyl 8-(2-[2-(2-ethylcyclopropyl-methyl)-cyclopropylmethyl]-cyclopropyl)-octanoate 2-(2-methoxy)-ethoxyethyl *tris*-9,10,12,13,15,16-methyl-ene-octadecanoate
A_2CE	$CH_3(CH_2)_7$-1-$(CH_2)_7CH_2OCH_2CH_2OCH_2CH_2OCH_3$	2-(2-methoxy)-ethoxyethyl 8-(2-*n*-octylcyclopropyl)-*n*-octyl ether 2-(2-methoxy)-ethoxyethyl 9,10-methyleneoctadecyl ether
A_2S	$CH_3(CH_2)_{16}COOCH_2CH_2OCH_2CH_2OCH_3$	2-(2-methoxy)-ethoxyethyl octadecanoate 2-(2-methoxy)-ethoxyethyl stearate
MC	$CH_3(CH_2)_7$-1-$(CH_2)_7COOCH_3$	methyl 8-(2-*n*-octylcyclopropyl)-octanoate methyl 9,10-methyleneoctadecanoate
C_2	$CH_3(CH_2)_4$-1-CH_2-1-$(CH_2)_7COOH$	8-[2-(2-*n*-pentylcyclopropylmethyl)-cyclopropyl]-octanoic acid
A_2	$CH_3OCH_2CH_2OCH_2CH_2OH$	2-(2-methoxy)-ethoxyethanol
A_1Hex	$CH_3(CH_2)_4COOCH_2CH_2OCH_3$	2-methoxyethyl hexanoate

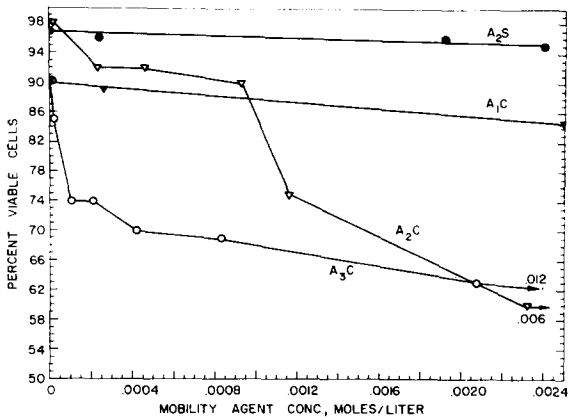

Fig. 1. Viability of lymphocytes treated with membrane mobility agents. Lymphocyte suspensions were incubated with the agents for 60 min (for details of the procedure, see Fig. 2). The viability of the cells thus incubated was evaluated by the dye-exclusion test, using trypan blue.

Escherichia coli cells are replaced to a considerable extent by cyclopropane acids after rapid growth has ceased [15]. An alkyl group containing oxygens was utilized in most agents as the hydrophilic chain (Table I). Dispersibility of the agent is a technical advantage in its use and this property varies with the hydrophilic chain chosen. Other atoms (nitrogen, phosphate-phosphorus) are also found in the hydrophilic chains of natural phospholipids, and will also be tried in membrane mobility agents.

Cytotoxicity measurements on lymphocytes with these compounds by the dye-exclusion test indicated a considerable dependence of toxicity on structure (Fig. 1). Certain compounds such as A_2C were toxic at concentrations considerably above that at which maximum effect on mobility was observed. Some compounds (e.g. A_3C) showed toxicity with amounts close to that at which an effect on mobility was seen.

Cap formation was followed on rabbit lymphocytes preincubated for various periods of time with one of the compounds, then treated with fluorescent antibody. At least twenty minutes preincubation with the aqueous dispersion of agent A_2C were required to produce a change in the observed rate from that of the control and between 1 and 2 h incubation was required for maximum response. A 1-h period was utilized in most experiments.

The rate of cap formation for cells treated with A_2C is compared with that of the corresponding controls in Fig. 2. Two points should be noted. First, the rate of cap formation in the treated cells is as much as 50 % higher than that of the controls. Second, extrapolation to zero cap formation time reveals that the treated cells might be initially more "prepared" to form caps than the control cells. The mobility agent may affect the first stage of cluster formation as well as the subsequent cluster movement to result in caps.

Inherent "high" or "low" capacity for cap formation does not alter the effects produced by the mobility agent (see Fig. 2).

The effects of most of the compounds listed in Table I were examined after a standard incubation period. Considerable variation in effectiveness as a membrane

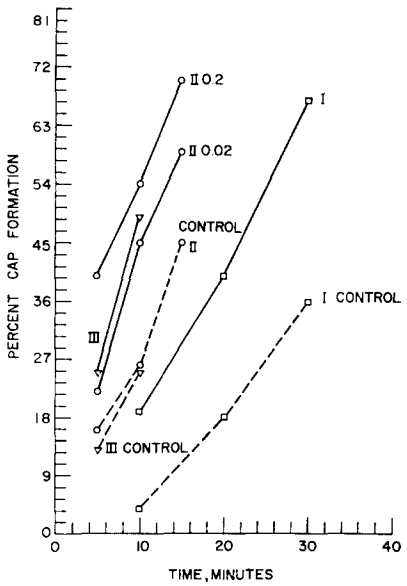

Fig. 2. Cap formation in lymphocytes treated with A_2C. Rabbit blood was anticoagulated with heparin (200 I.U./cm^3) and diluted with NaCl–phosphate buffer, pH 7.3 (137 mM NaCl–1.8 mM KH_2PO_4–8.2 mM Na_2HPO_4) to 40 % of original volume. Diluted blood was layered over sodium metrizoate (Nyegaard, Oslo, Norway)–Ficoll (Pharmacia, Uppsala, Sweden) mixture (containing 6.3 % Ficoll and 10 % sodium metrizoate), and centrifuged for 30 min at 400 × g at 25 °C. Cells collected from the interphase contained over 80 % lymphocytes. Cells were centrifuged, washed 3 times in buffer containing albumin and resuspended in the same or A_2C buffer. (A_2C was suspended in buffer (with Vortex) and albumin added). Final concentrations: lymphocytes 10^7 cells/cm^3, A_2C 0.02–0.2 μl/cm^3, 0.2 % albumin, 5 mM glucose. Usual sample volume: 0.2 cm^3. The cell suspension was incubated at 22 or 24 °C for 60 min. Fluorescein-labeled goat anti-rabbit Ig antibodies (source: rabbit IgG injected into goat, Ig purified from antiserum by affinity chromatography on rabbit IgG–Sepharose 4B, and conjugated with fluorescein isothiocyanate were then added to cells (0.05 mg per $2 \cdot 10^6$ cells) and incubation continued. Samples were treated at intervals with cold 10 mM NaN_3 in buffer–albumin, cells centrifuged and washed with same buffer, suspended in buffered glycerol and counted under a Zeiss fluorescence microscope. Three separate experiments are shown to illustrate the differences observed between control cells exhibiting various rates of cap formation and A_2C-treated cells. Expt I: 0.1 μl A_2C per cm^3; (log concn −3.7); temp. 22 °C. Expt II: 0.02 and 0.2 μl A_2C per cm^3; temp. 24 °C. Expt III: 0.2 μl A_2C per cm^3; temp. 24 °C.

mobility agent was observed. Of the hydrophilic chains, A_2 ($CH_3OCH_2CH_2OCH_2C_2$–) gave rise to compounds with the highest activity. Cap formation after treatment with CH_3, A_1, A_2 and A_3 esters of the C acid is illustrated in Fig. 3A. One, two or three cyclopropane rings at the hydrocarbon end of the hydrophobic C_{18} chain do not differ greatly in effect on agent activity (Fig. 3B). The ether, A_2CE, was less than the ester A_1C in effect. Other compounds listed in Table I exhibited little or no effect on cap formation.

Bovine serum albumin raises the concentration level of agent required for affecting mobility by a factor of 5–10. However, the shape of the dose–response curve is not changed and we believe therefore that the albumin combines with agent.

The detailed mechanism of surface receptor motion and its role in the transfer of external signals to the interior of the cell has not yet been finally settled [16–18].

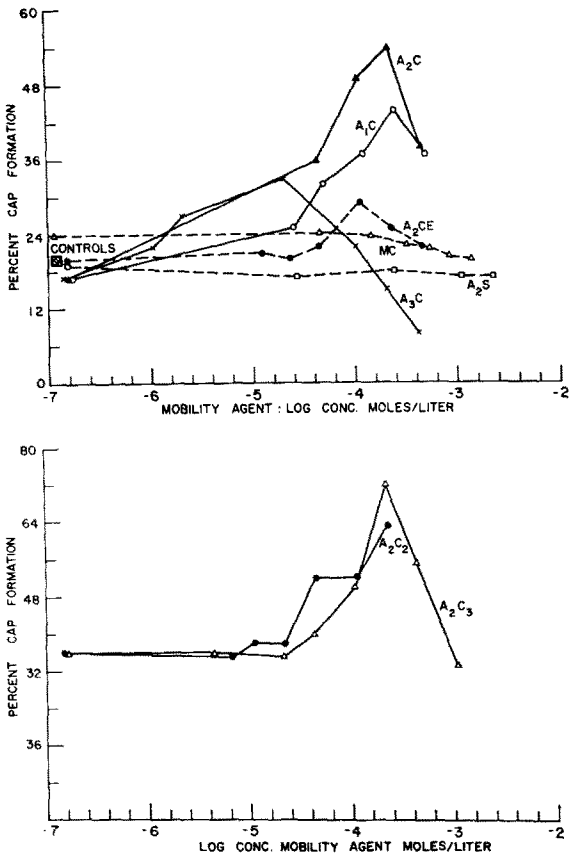

Fig. 3. Cap formation in lymphocytes as a function of amount of added mobility agent. (See caption. Fig. 2, for experimental details). A. Cells were incubated with the indicated concentrations of various compounds (for key to symbols, see Table 1) for 60 min at 22–24 °C, fluorescent-labeled antibody added and reaction stopped after 10 min as described in caption, Fig. 1. ○—○, A_1C; ▲—▲, A_2C; ×—×, A_3C; △- - - △, MC; ●- - -●, A_2CE; □- - -□, A_2S. B. Comparison of cap formation promoted by agents with two and three cyclopropane rings. (See captions, Figs 1 and 2a for details). Reaction stopped after 12 min. ●—●, A_2C_2; △ - △, A_2C_3.

Recently observed differences in membrane mobility between differentiated and non-differentiated cell types [19] imply the possibility that mobility of sites is helpful to the progress of the cell response to stimuli.

Membrane mobility agents offer a useful way of perturbing usual membrane processes. Additional variations in the structure of the mobility agents and investigation of their physical-chemical properties and the mechanism by which they affect membrane processes will be of interest.

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