G_t, which binds to mutants CD2 and EF1, is not measured directly in these experiments. Hypothetically, the G_t nucleotide binding site may be unoccupied or may contain GDP or GTP. We find it likely that the GDP form of G_t binds to photoactivated rhodopsin, and that the release of GDP then allows MII stabilization (*12*). The activation step that is blocked in mutants CD2 and EF1 may be the signal that induces formation of the GTP binding pocket in G_t. Thus, we speculate that mutants CD2 and EF1 bind to G_t that is nucleotide free.

Many receptors that couple to G proteins have been identified, and deciphering the mechanism of G protein activation is central to an understanding of G protein–mediated signal transduction. Mutants of the β_2-adrenergic receptor (β-AR), which is structurally related to rhodopsin (*13*), have been studied in whole-membrane preparations with steady-state agonist binding assays. A 34–amino acid deletion from the third cytoplasmic loop (EF) of β-AR produced a receptor with a single high-affinity agonist state, which did not activate adenylate cyclase (*14, 15*). The interpretation of this result was that the mutant receptor was uncoupled from the G protein, G_s. A seven–amino acid deletion in the same loop (EF) of β-AR results in a moderate impairment of the adenylate cyclase response and a single high-affinity agonist state (*16*). It was concluded that this receptor domain in loop EF might participate in the transmission of an agonist-induced stimulatory signal to G_s. Our direct demonstration of an inactive mutant rhodopsin-G_t complex supports the interpretation that these deletion mutants of β-AR bound G_s to form a complex with impaired activity. Furthermore, the combined results of our and other studies (*14–17*) suggest that the G_s-induced high-affinity agonist state of β-AR is analogous to the G_t-induced stabilization of MII. The correlation between rhodopsin and β-AR might be tested further by purifying mutant β-ARs and studying agonist binding affinity and the stimulation of G_s in artificial vesicles. Thus, the study of rhodopsin-G_t interactions may provide general information regarding transmembrane signaling, and our direct spectroscopic assay of purified rhodopsin mutants should allow a more detailed analysis of discrete steps in the G protein activation pathway.

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9. Solubilization of rhodopsin in steroid-containing micelles allows MII formation, G_t binding, and G_t activation and release to be monitored separately (*1, 10*). The light-induced intermediates, metarhodopsin I (MI) and metarhodopsin II (MII), exist in equilibrium, and binding of G_t to MII shifts the equilibrium toward MII. The amount of MII-G_t complex and, therefore, the binding of G_t to MII can be quantitated by measuring the G_t-dependent shift from MI (λ_{max} = 480 nm) to MII (λ_{max} = 380 nm). The resulting MII-G_t complex remains stable for minutes in the absence of GTP and the kinetics of its formation and steady-state concentration can be determined. However, in the presence of GTP, MII stimulates the exchange of GDP for GTP on G_t. The GTP-bound form of the α subunit of G_t then dissociates from MII and the equilibrium shifts back to MI [D. Emeis and K. P. Hofmann, *FEBS Lett.* **136**, 201 (1981)].
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28. Transiently transfected COS-1 cells (~10^8) treated with 11-*cis*-retinal were solubilized in buffer A [7 mM deoxylysolecithin, 5 mM cortisone, 10 mM Hepes (pH 8), and 1 mM $CaCl_2$] supplemented with 1 mM phenylmethylsulfonyl fluoride at room temperature for 1 hour. Insoluble material was separated by centrifugation at 100,000*g* for 30 min at 20°C, and pigment was purified by an immunoaffinity procedure (*8*), with the exception that washing and peptide elution were performed in buffer A. UV-visible spectra of rhodopsin and mutants CD1, CD2, and EF1 were identical (visible λ_{max} = 500 nm). In the flash photolysis experiments, the effective path length was 0.7 cm on 0.8 ml of sample that contained pigment (200 nM), G_t (40 nM), and GTP (20 μM) in buffer A. Each light flash (520 ± 15 nm) bleached 5% of the pigment present. The formation of MII was measured as the difference between absorbances at 380 nm and 417 nm (the isosbestic point between MI and MII). Experiments were performed at 4°C, but for each sample, a separate experiment was performed with pigment alone at 20°C. At the higher temperature, the normal extent (60%) of conversion to an MII species was observed for each mutant tested. COS cell rhodopsin was identical to both washed disk membrane and purified solubilized bovine rhodopsin with respect to MII formation, extra MII formation in the presence of G_t, and the absence of the extra MII signal in the presence of G_t and GTP (R. R. Franke, B. König, T. P. Sakmar, H. G. Khorana, K. P. Hofmann, unpublished data).

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Technical Comments

Inhibition of HIV-1 Infectivity by Phosphate-Methylated DNA: Retraction

We recently reported (*1*) that phosphate-methylated 20-nucleotide DNA oligomers are able to inhibit human immunodeficiency virus type–1 (HIV-1) infectivity through hybridization to the viral RNA or integrated viral DNA. The oligomers we used to test the biological activity of phosphate-methylated DNA with regard to the inhibition of HIV-1 infectivity were synthesized according to the method of Moody *et al.* (*2*) in which methylation of natural oligomers is achieved by a three-step process.

After the publication of our report (*1*), we analyzed our original samples of phosphate-methylated DNA with reversed-phase C18 and strong anion exchange (SAX) high-performance liquid chromatography (HPLC). Analysis by reversed-phase C18 HPLC is largely based on a difference in polarity between compounds, while the elution order in SAX HPLC is dictated for the most part by the negative charge of the compounds. Therefore, completely or partially phosphate-methylated DNA oligomers are expected to elute before their natural DNA counterparts in SAX HPLC, while for the reversed-phase C18 HPLC the order of elution is reversed. A reversed-phase C18 chromatogram and a SAX chromatogram of a sample of the phosphate-methylated antisense (−) NEF sequence are shown in Fig. 1.

We had assumed that the first peak in the SAX profile represented the completely phosphate-methylated oligonucleotide sequence. However, treatment of part of the sample with tert-butylamine/water (1:1 v/v) for 16 hours at 50°C [which causes complete demethylation of methyl phosphotri-

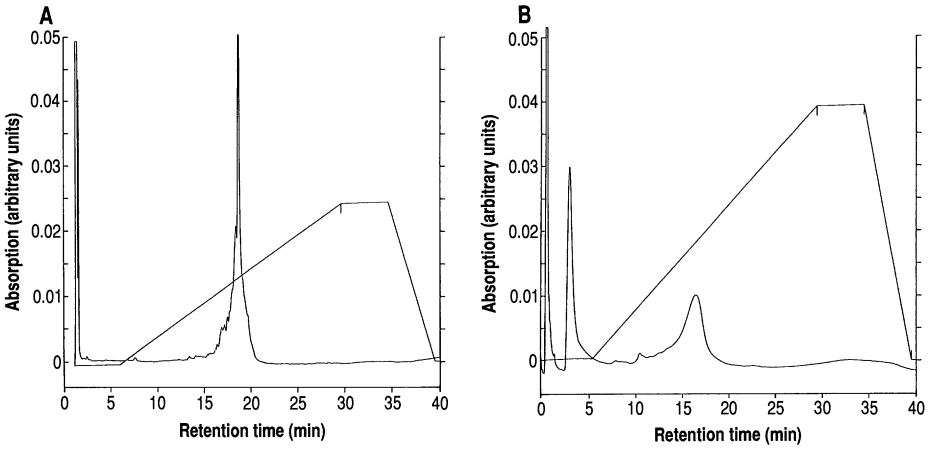

Fig. 1. (**A**) Reversed-phase C18 HPLC chromatogram of the phosphate-methylated (−) NEF sequence. Simultaneous injection with the natural DNA counterpart indicates that the peak with an elution time of 19 minutes consists of natural DNA. The first peaks (at 1 to 2 minutes) correspond to highly polar components. (Zorbax ODS column; detection at 254 nm; flow rate, 1.0 ml/min. Buffer A: 0.1 M triethylammoniumacetate (TEAA) (pH = 7.0, 5% acetonitril, v/v); buffer B: 0.1 M TEAA (pH = 7.0, 50% acetonitril, v/v); gradient: 0 to 5 minutes isocratic 100% A, 5 to 30 minutes linear 0 to 50% B). (**B**) SAX HPLC profile of phosphate-methylated (−) NEF. The peak at 16.5 minutes corresponds to natural DNA. We attribute the first peaks (0 to 5 minutes) to non-DNA compounds. Self-packed Zorbax SAX column, detection at 254 nm, flow rate 1.0 ml/min. Buffer A: 0.001 M KH_2PO_4 (pH = 6.5, 30% acetonitril, v/v); buffer B: 0.3 M KH_2PO_4 (pH = 6.5, 30% acetonitril, v/v); gradient: 0 to 5 minutes isocratic 100% A, 5 to 30 minutes linear 0 to 80% B.

ester systems (*3*)] showed almost no change in the SAX chromatogram or in the reversed-phase C18 chromatogram. The other constructs we reported (*1*) showed comparable HPLC profiles, with varying amounts of natural DNA and highly polar compounds. In order to determine the identity of all the compounds in the mixture, we repeated the synthesis protocol for the "phosphate-methylated" sense (+) TAR sequence and separated the mixture into fractions by different extraction and precipitation steps. Analysis by reversed-phase C18 and SAX HPLC and proton nuclear magnetic resonance (NMR) spectroscopy revealed that the highly polar compounds were pyridinium or triethylammonium salts of *p*-toluenesulfonic acid (compounds derived from the *p*-toluenesulfonyl chloride–mediated esterification step). Large amounts of natural DNA, but no phosphate-methylated DNA, were detected (the detection limit being approximately 0.01%). There is a large difference between the retention times of phosphate-methylated DNA and natural DNA on both reversed-phase C18 and SAX HPLC, which we observed when we used well-characterized short phosphate-methylated oligonucleotides.

From these data we conclude that the samples we used for the inhibition experiments (*1*) contained neither completely nor partially phosphate-methylated DNA, but only natural DNA and several by-products of the synthesis (pyridinium and triethylammonium salts of *p*-toluenesulfonic acid). Since some of these compounds (particularly pyridinium) displayed considerable ultraviolet absorption (at a wavelength of 260 nm, extinction coefficients 5×10^3 liter mol^{-1} cm^{-1} for pyridinium and 10×10^3 liter mol^{-1} cm^{-1} per nucleotide unit), the reported inhibitory concentration values are considerably higher than the actual values for DNA. Moreover, there is a great variation in the ratio between the amount of natural DNA and the amount of polar by-products in the samples. This result contrasts with earlier statements (*1, 2*) that the degree of phosphate methylation of the tested DNAs was 90 to 100%.

It appears that the base-protection step in our synthesis protocol yielded an almost insoluble product, which caused low yields in the next steps and prevented adequate characterization of the intermediate products. In view of the composition of the samples, we now believe that our hybridization studies (*1*) of the longer (more than nine nucleotides) phosphate-methylated DNA oligomers with complementary natural DNA (by means of ultraviolet spectroscopy) and with yeast phenylalanine transfer RNA (by means of NMR spectroscopy) do not warrant the interpretation given in our report (*1*). There is no evidence to suggest that the observed antiviral effects should be ascribed to the phosphate methylation of natural DNA.

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