G₈, which binds to mutants CD2 and EF1, is not measured directly in these experiments. Hypothetically, the G₈ nucleotide binding site may be unoccupied or may contain GDP or GTP. We find it likely that the GDP form of G₈ 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 G₈. Thus, we speculate that mutants CD2 and EF1 bind to G₈ 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 β₂-adrnergic 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, G₈. 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 G₈. Our direct demonstration of an inactive mutant rhodopsin-G₈ complex supports the interpretation that these deletion mutants of β-AR bound G₈ to form a complex with impaired activity. Furthermore, the combined results of our and other studies (14-17) suggest that the G₈-induced high-affinity agonist state of β-AR is analogous to the G₈-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 G₈ in artificial vesicles. Thus, the study of rhodopsin-G₈ interactions may provide general information regarding transmembrane signaling, and our direct spectroscopic assay of purifed rhodopsin mutants should allow a more detailed analysis of discrete steps in the G protein activation pathway.

Technical Comments

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

We recently reported (1) that phosphatase-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 phosphatase-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 phosphatase-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 phosphatase-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 phosphatase-methylated antisense (5′-NEF sequence are shown in Fig. 1.

We had assumed that the first peak in the SAX profile represented the completely phosphatase-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 phosphori-
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 \times 10^2 \text{ liter mol}^{-1} \text{ cm}^{-1} \text{ for pyridinium and } 10 \times 10^3 \text{ liter mol}^{-1} \text{ cm}^{-1} \text{ 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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REFERENCES AND NOTES
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