PERSPECTIVES: ATMOSPHERIC CHEMISTRY

A Marine Source for Alkyl Nitrates

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It has long been known that halogenated methanes and ethanes detected in the atmosphere have substantial natural sources. Indeed, the most abundant organohalogen, methyl chloride, with a concentration of ~550 parts per trillion by volume (pptv), comes mostly from natural sources. Methyl chloride constituted 15% of the primary sources of chlorine entering the stratosphere in the early 1990s; 82% were entirely of human origin.

Chuck *et al.* now report the surprising observation that methyl nitrate and ethyl nitrate, the first two members of the large group of alkyl nitrates, may also have a substantial natural regional source in the marine environment. On page 1151 of this issue, they present measurements of methyl and ethyl nitrate in air and surface water of the Atlantic Ocean that provide strong evidence for a natural origin (*1*). These compounds are normally considered as purely anthropogenic emissions.

Organic nitrates, $RONO_2$, the organic esters of nitrous acid, have long been used as explosives. For example, the explosive nitroglycerin is a triester of glycerin and nitrous acid. Methyl nitrate itself is highly explosive. Organic nitrates are also widely used as vasodilatoric drugs against angina pectoris.

A key source of alkyl nitrates in the atmosphere is the light-driven chemistry of air pollution. Reaction of $\bullet OH/O_2$ during the day or $\bullet NO_3/O_2$ during the night with a C-H or C-C bond of an alkane or alkene leads to the formation of peroxyalkyl radicals, $RO_2\bullet$. In NO-rich air, such radicals mainly react to form nitrogen dioxide and alkoxy radicals:

$$RO_2\bullet + NO\bullet \rightarrow RO\bullet + NO_2\bullet \quad (1)$$

ultimately leading to carbonyl compounds rather than alkyl nitrates. But in a side reaction, presumably via peroxyalkyl nitrites RO_2NO as intermediates, alkyl nitrate compounds are formed:

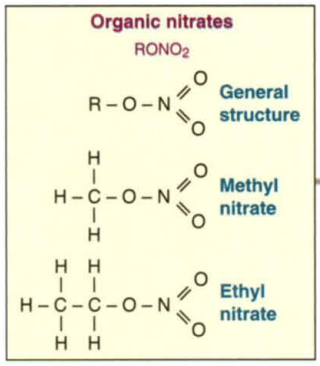
$$RO_2\bullet + NO\bullet\ (+M) \rightarrow RONO_2\ (+M) \quad (2)$$

The yield of the second reaction increases from less than 0.014 for ethane to 0.33 for octane. For methane, it is considered to be close to zero. So far, no demonstrated pathway can account for the measured levels of methyl nitrate in air.

At high concentrations of NO_2 and in the lower stratosphere, a third reaction becomes important, particularly for the formation of methyl nitrate (*2*):

$$RO\bullet + NO_2\bullet \rightarrow RONO_2 \quad (3)$$

Organic peroxy radicals $RO_2\bullet$ may also react with each other at rates that can compete with reactions involving NO and $HO_2\bullet$, particularly when NO/NO_2 concentrations are low, as is the case in the clean marine atmosphere. The primary sources of alkyl nitrates are therefore in NO_x-rich (i.e., urban or traffic-affected) regions.



Sources of organic nitrates.

The atmospheric lifetime of alkyl nitrates is long enough to undergo long-range transport in the troposphere and mix with regions of clean air (*3*). Alkyl nitrates with up to 17 carbon atoms have been identified in continental and marine air (*4*). A broad spectrum of alkyl nitrates, some with a second functionality (a nitrooxy or hydroxy group), has been detected in air above the North and South Atlantic (*5*). Organic nitrates with a carbonyl function deriving from reaction with isoprene are also found in air (*6*).

The formation of most simple alkyl nitrates is still an open problem. The sources of methyl nitrate in continental air remain obscure (*7*). Besides Reactions 2 or 3, a further source could be the degradation of the pollutant peroxyacetylnitrate $[CH_3C(O)OONO_2]$ to CH_3ONO_2 and CO_2 (*8*). Reaction of methanol with nitric acid on aerosols may also result in higher yields of methyl nitrate in continental and polluted air (*9*).

However, all these reactions cannot explain the high concentrations observed in the tropical Pacific; hence, a substantial marine source for methyl and ethyl nitrate should exist, although a background level of ethyl nitrate can derive from Reaction 2 (*10*). Support for this assertion comes from recent measurements of methyl nitrate profiles above the South Pacific. In the marine boundary layer, concentrations of up to 50 pptv near Christmas Island and 20 to 35 pptv near Western Samoa were reported (*11*). Concentrations of 30 to 80 pptv for methyl nitrate in air were measured at Neumayer research station in Antarctica (*12, 13*). The station is close to the highly bioactive waters of the Antarctic Ocean.

Chuck *et al.* now report measurements of methyl and ethyl nitrate in seawater and air samples along two Atlantic Ocean transects (*1*). They find surface waters at the equator to be highly supersaturated for both species. The study provides the first direct evidence that regions in the oceans exist that are a definite source for the two short-chain nitrates.

Several reaction pathways may be involved in the formation of these species in ocean water. First, a form of activated nitrate similar to silver nitrate can react quantitatively with alkyl bromides and iodides, found as natural products in the marine environment, to form the respective nitrates. Second, alkylation of the nitrate ion may occur, similar to the methylation of chloride. Third, methyltransferases could provide a biochemical pathway of methylation. Methyl donors may include *S*-adenosylmethionine and derivatives of tetrahydrofolic acid, which are known as methyl donors in cells (*14*).

The chemistry of many complex molecules is well understood. More sur-

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prising is the fact that even for very simple compounds like methyl and ethyl nitrate, basic questions of their chemistry in the environment are still open.

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PERSPECTIVES: BIOMEDICINE

Contact—How Platelets Touch von Willebrand Factor

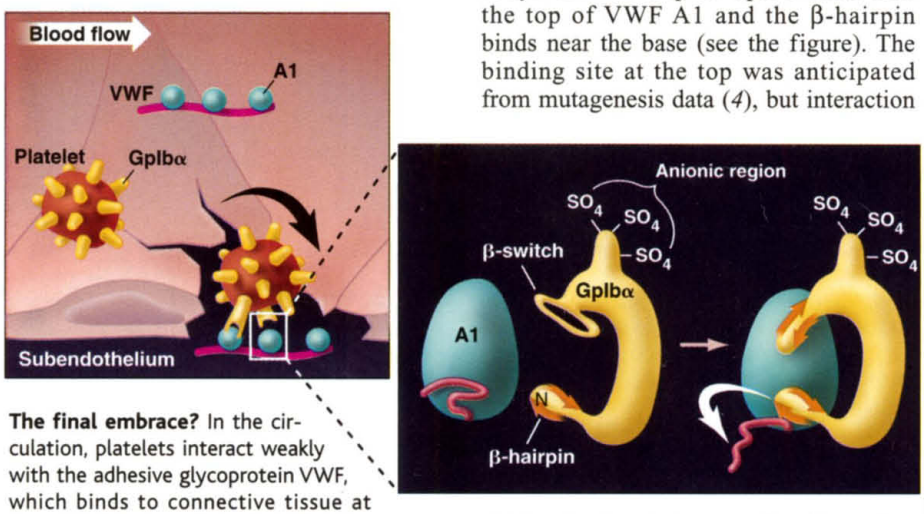
J. Evan Sadler

We cannot live without platelets, the small anucleate blood cells that aggregate to seal leaks at sites of vascular injury. Plasma von Willebrand factor (VWF) acts as an extracellular adapter in this process, binding to collagen in the wall of damaged blood vessels and then to membrane glycoprotein Ibα (GpIbα) on the platelet surface (*1*, *2*). Bleeding ensues when this interaction cannot occur, and fatal thrombosis (thrombotic thrombocytopenic purpura) follows when it cannot be terminated by feedback proteolysis of VWF. The kinetic properties of the binding of VWF to GpIbα have evolved to satisfy some rather special requirements that make platelet adhesion possible. On page 1176 of this issue, Huizinga *et al.* (*3*) give us our first look at the structural features that underlie this life-saving interaction.

VWF is a multimeric protein composed of identical 250-kD subunits, with the multimer typically exceeding 10,000 kD. Each subunit has a single 24-kD A1 domain that binds to the amino-terminal 45-kD segment of platelet GpIbα (see the figure). Platelet GpIbα is the largest component of a cell surface complex that contains at least three other membrane proteins. The affinity of binding appears to depend on the assay conditions. Soluble VWF and platelets do not readily interact in the blood, but when platelets flow across a VWF-coated surface, they adhere rapidly and begin to roll along the surface. Reversible rolling provides enough interaction time for other kinetically slow receptors to engage their ligands and initiate stable platelet adhesion and activation. The VWF-dependent capture and transient tethering of platelets is most efficient at the relatively high fluid shear rates found in small arterioles. Platelet adhesion therefore behaves as though it is regulated by the surface adsorption of VWF and by fluid shear stress (*1*).

These observations often are interpreted as evidence that immobilization or shear stress induces conformational changes in VWF that increase its affinity for GpIbα. This model is consistent with a surprising phenotype caused by certain mutations in either protein. von Willebrand disease (VWD) type 2B is a bleeding disorder characterized by increased binding of mutant VWF to GpIbα. The type 2B mutations are clustered in a small patch on the VWF A1 domain, which is remote from the GpIbα binding site that has been localized by mutagenesis (*4*). The clinically similar "platelet-type pseudo-VWD" is caused by mutations in the amino-terminal region of GpIbα that increase its affinity for VWF (*5*, *6*). Thus, low-affinity conformations of both the VWF A1 domain and GpIbα appear to be maintained by inhibitory mechanisms that are relieved by constitutive gain-of-function mutations. Whether shear stress or surface binding causes similar conformational changes remains controversial, and the behavior of adhering platelets can be modeled satisfactorily without invoking shear-dependent changes in affinity (*7*).

Against this background, the structure of the VWF A1-GpIbα complex (*3*) has remarkable explanatory power. To facilitate crystallization, Huizinga and colleagues engineered high-affinity variants: VWF A1 carried the VWD type 2B mutation R543Q, and the GpIbα fragment carried the platelet-type pseudo-VWD mutation M239V. The GpIbα fragment consists of eight leucine-rich repeats (LRRs) that form an elongated curve. The LRR framework is flanked by an amino-terminal β-hairpin motif and by a more complex carboxyl-terminal region with a protruding loop. The concave face of GpIbα grabs VWF A1 in a pincer-like grip: The carboxyl-terminal loop of GpIbα binds near the top of VWF A1 and the β-hairpin binds near the base (see the figure). The binding site at the top was anticipated from mutagenesis data (*4*), but interaction



The final embrace? In the circulation, platelets interact weakly with the adhesive glycoprotein VWF, which binds to connective tissue at sites of injury. At sufficiently high fluid shear rates, platelets bind to VWF and exhibit rolling adhesion. Each VWF subunit of the multimer has a single A1 domain (blue) that binds to platelet GpIbα (yellow). In the free A1 domain, an amino-terminal extension (pink) appears to block a binding site for the amino-terminal β-hairpin (orange arrows) of GpIbα. Binding requires the amino-terminal extension of A1 to move, and also induces the β-switch (yellow loop) of GpIbα to form a β-strand motif (orange arrows). The anionic sulfated region of GpIbα (closer to the carboxyl-terminal than the β-switch) does not directly contact the VWF A1 domain.

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