pAcPstJ contains *hr*2, which includes eight palindrome sequences distributed over about 1 kb of DNA (*3*). pAcPstJ replicated at high levels relative to the other plasmids (Fig. 2C). PUC19 DNA did not replicate when transfected into AcMNPV-infected Sf9 cells, and pAcPstJ did not replicate when transfected into uninfected cells. Quantification of Dpn I–resistant hybridization signals indicated that plasmids with more palindromes replicated more efficiently than those with fewer palindromes (Fig. 1A).

Deletion analysis of sequences that specify AcMNPV infection–dependent DNA replication demonstrated that a sequence containing a single complete palindrome was capable of promoting replication. Because sequences containing multiple palindromes elicit a disproportionate stimulation of DNA replication, the full complement of palindromes may be essential for optimal replication. Although the relation of the *hr*-dependent plasmid replication to in vivo viral DNA replication is not presently known, the distribution of *hrs* at intervals around the genome suggests that they may act cooperatively to accelerate the replication of the genome from as many as six different sites. Although palindrome sequences are common features of replication origins in bacteria, plasmids, phage, and eukaryotic viruses (*6*), the distribution and repetitious nature of these AcMNPV candidate-origins appear to be unusual.

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Genesis of Acetate and Methane by Gut Bacteria of Nutritionally Diverse Termites

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The evolution of different feeding guilds in termites is paralleled by differences in the activity of their gut microbiota. In wood-feeding termites, carbon dioxide–reducing acetogenic bacteria were found to generally outprocess carbon dioxide–reducing methanogenic bacteria for reductant (presumably hydrogen) generated during microbial fermentation in the hindgut. By contrast, acetogenesis from hydrogen and carbon dioxide was of little significance in fungus-growing and soil-feeding termites, which evolved more methane than their wood- and grass-feeding counterparts. Given the large biomass of termites on the earth and especially in the tropics, these findings should help refine global estimates of carbon dioxide reduction in anoxic habitats and the contribution of termite emissions to atmospheric methane concentrations.

Although generally recognized for their ability to thrive on a diet of wood, the feeding behavior and nutritional ecology of termites is quite diverse and not limited to xylophagy. Some species forage for grass and leaves, whereas others feed exclusively on soil, presumably deriving nutrition from the humic compounds therein (*1*, *2*). Still others cultivate and consume cellulolytic fungi, which, when ingested with plant materials, augment the digestive enzymes of the insect (*3*). Nevertheless, all known termites have a dense and diverse hindgut microbial community, which aids in digestion and which is the source of fermentation products such as acetate, methane (CH_4), and H_2 (*4*).

The symbiotic hindgut microflora of wood-eating, "lower" termites (for example, *Reticulitermes flavipes*) includes protozoa and bacteria and effects an essentially homoacetic fermentation of wood polysaccharide (principally cellulose) consumed by the insect. Cellulolytic protozoa first hydrolyze cellulose and ferment each glucose monomer to acetate, carbon dioxide (CO_2), and H_2 (*5*):

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ CO_2 + 4\ H_2$$

then CO_2-reducing acetogenic bacteria convert H_2 and CO_2 to an additional acetate molecule (*6*):

$$4\ H_2 + 2\ CO_2 \rightarrow CH_3COOH + 2\ H_2O$$

The three net acetates formed per glucose monomer are absorbed from the hindgut and oxidized by the termite to support up to 100% of the insect's respiratory requirement (*7*):

$$3\ CH_3COOH + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

Both H_2 and CH_4 (the latter formed by reduction of CO_2 by methanogenic bacteria) are also emitted by termites, and the extent to which termites contribute to global increases in atmospheric CH_4 has been hotly debated (*8*–*14*). However, emission of these gases represents only a small part of reduction equivalents ($H^+ + e^-$) generated by microbial fermentation in the hindgut of *R. flavipes* (*7*). In limited studies of other wood-eating "lower" and "higher" termites (the latter of which contain only bacteria in their hindguts), a similar pattern was observed (*6*). This in itself was surprising, because, in most anoxic habitats low in sulfate and nitrate, CO_2 reduction to CH_4 (not acetate) is the dominant H_2-consuming process (*15*). Therefore, to determine whether bacterial acetogenesis, rather than methanogenesis, was the major H_2-consuming "electron sink" reaction of hindgut fermentation of termites in general, we examined a variety of tropical species representing different feeding guilds (different patterns of food resource preference). Because opportunities to collect fresh specimens of many of the species (especially those from remote regions) were rare, our sampling strategy in the time available was to maximize species diversity within a particular feeding guild rather than to sample repeatedly a given species from different sites for replicate analyses. Included in this study were wood-feeding members of three families of "lower" termites (Hodo-, Kalo- and Rhinotermitidae), and wood-, grass-, and soil-feeding and fungus-growing representatives of the higher termite family Termitidae, which includes about three-quarters of all known termite species (*1*, *2*).

We quantified acetogenesis from CO_2 by measuring the reduction of $^{14}CO_2$ to ^{14}C-acetate by anoxic suspensions of termite gut contents. This was done under two conditions: (i) in the presence of exogenously

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supplied H_2 and (ii) with reductant (presumably H_2) produced endogenously by gut microbes present in the suspension (6). By contrast, CO_2-reducing methanogenesis by the gut microbiota was usually estimated as CH_4 emission from live termites during brief (2 to 4 hours) incubation in stoppered bottles (7). This latter technique is sensitive and noninvasive and minimizes disruption of the insects and their gut microbiota, and it was well suited to measurements in the field (16).

Rates of acetogenesis from CO_2 (with endogenous H_2) for 14 wood-feeding termites and one grass-feeding species were, on average, three times those of CH_4 emission (Table 1) (17, 18). However, this assay condition may seriously underestimate in situ rates of acetogenesis from CO_2, because homogenization and dilution of gut contents probably disrupt important physical interactions between H_2-producing microbes and H_2-utilizing acetogenic bacteria that would otherwise occur in situ (19). Not surprisingly then, rates of acetogenesis from CO_2 by wood-feeding termites usually increased to more than ten times those of CH_4 emission when acetogenesis was measured in the presence of exogenously supplied H_2. By contrast, for both fungus-growing and soil-feeding termites, rates of CH_4 emission were always greater than rates of acetogenesis from CO_2, even when the latter process was measured in the presence of exogenously supplied H_2.

Differences in acetogenesis and methanogenesis activity between termites of different feeding guilds were also apparent. Rates of CO_2 reduction to acetate by gut contents from wood- and grass-feeding termites (with or without exogenously supplied H_2) were greater than those of fungus-growing or soil-feeding termites (Table 1) (18). By contrast, rates of CH_4 emission by soil-feeding and, to a lesser extent, fungus-growing termites were greater than those of almost all wood-feeding termites. Gut contents from all lower and higher wood-feeding termites (and from one grass-feeding species) displayed readily detectable levels of CO_2-reducing acetogenic activity, whereas one fungus-growing species and five soil-feeding species exhibited almost no acetogenesis from $H_2 + CO_2$, even when supplied with exogenous H_2. Conversely, all fungus-growing and soil-feeding species evolved relatively high amounts of CH_4, but three wood-feeding species (*C. formosanus*, *C. cavifrons*, and *P. occidentis*) evolved little or none.

It might be argued that the relatively low rate of CH_4 emission from wood-feeding termites is due to aerobic oxidation of CH_4 before it emanates from the insect. However, kinetic analyses of O_2 consumption by live termites suggest that this is not the case (7). Moreover, we have measured rates of $^{14}CO_2$ reduction to $^{14}CH_4$ by anoxic gut contents from the wood-feeding *R. flavipes*, *Z. angusticollis*, and *N. nigriceps* (6) (see also Table 1), as well as from *M. parvus*, *N. lujae*, and the soil-feeding *C. speciosus* (20). In all cases, such rates were less than or equal to CH_4 emission by live termites, even when the gut contents were supplied with exogenous H_2. Unfortunately, because of limited time and supplies in the field, we were unable to determine rates of $^{14}CO_2$ reduction to $^{14}CH_4$ by gut contents from other soil-feeding species or from fungus-growing species.

It is not surprising that animals with anaerobic, fermentative microbial communities in their alimentary tract evolve CH_4. A classic example is a bovine animal, whose rumen microbiota evolves up to 200

Table 1. Rates of $H_2/^{14}CO_2$ acetogenesis by termite gut contents and CH_4 emission by live termites of different feeding guilds. Units are micromoles of product per gram of termite per hour. The origin and condition of termites before the assay are as indicated in (17). The first six species listed are "lower" termites, the others are "higher" termites [see (1)]. The standard assay system has been described in detail (6) and is only summarized here. Guts from 20 to 60 worker termites were removed in an anaerobic chamber and were pooled in an anoxic, buffered salt solution before homogenization. Reaction vials (8-ml) had a final liquid volume of 0.5 ml and contained 1.2 μmol of $NaH^{14}CO_3$ (specific activity, ~6.5×10^4 dpm/μmol) and the equivalent of two to four homogenized termite guts. The atmosphere in the reaction vials consisted of 100% N_2 (for determination of rates of ^{14}C-acetate formation from $^{14}CO_2$ by endogenously produced H_2) or 100% H_2. After termination of the reaction, the supernatant fluid was analyzed for ^{14}C-labeled products by high-performance liquid chromatography. Modified assays, performed with gut homogenates of *R. flavipes* incubated with 52 mM $NaH^{14}CO_3$ in the liquid phase and 20% $^{14}CO_2$/80% N_2 (or 80% H_2) in the gas phase, gave results virtually identical to those tabulated for the standard assay system. Results are mean values of duplicate reactions of samples from the same pooled gut homogenate for $n = 1$ homogenate, except for the following species (for which the data are mean values of duplicate reactions for n as indicated): *R. flavipes*, $n = 20$; *Z. angusticollis*, $n = 3$; *M. parvus*, $n = 3$; *N. lujae*, $n = 2$; *C. albotarsalis*, $n = 2$; *C. speciosus*, $n = 3$. Values for *R. flavipes*, *P. simplex*, *Z. angusticollis*, *N. costalis*, and *N. nigriceps* were published as portions of a separate study (6) and are included here for comparison. The rate of H_2/CO_2 acetogenesis reported here for *R. flavipes* is slightly lower than the value reported previously, which was based on $n = 6$ (6). Assays of *C. cavifrons* were done by J. Klenz with J.A.B. during a summer course in microbial diversity at the Marine Biological Laboratory, Woods Hole, Massachusetts. For results where $n \geq 3$, results are mean values ± standard deviation (18).

Termite	^{14}C-acetate		CH_4 emitted*
	Exogenous H_2	Endogenous H_2	
	Wood-feeding termites		
Coptotermes formosanus	1.66	0.10	0.01
Cryptotermes cavifrons	1.34	0.58	0.00
Prorhinotermes simplex	1.18	0.57	0.45†
Pterotermes occidentis	2.07	0.48	0.00
Reticulitermes flavipes	0.93 ± 0.43	0.09 ± 0.06	0.10
Zootermopsis angusticollis	0.33 ± 0.25	0.07 ± 0.02	1.30
Amitermes sp.	5.16	1.03	0.13
Gnathamitermes perplexus	1.83	0.13	0.21
Microcerotermes parvus	4.96 ± 1.34	1.16 ± 0.98	0.14
Nasutitermes arborum	2.29	3.00	0.13
Nasutitermes costalis	5.96	0.99	0.49†
Nasutitermes lujae	1.91	0.13	0.15
Nasutitermes nigriceps	3.68	0.89	0.24
Tenuirostritermes tenuirostris	0.98	0.05	0.11
	Grass-feeding termite		
Trinervitermes rhodesiensis	2.70	2.38	0.18
	Fungus-growing termites		
Macrotermes mülleri	0.05	0.01	0.25
Pseudacanthotermes militaris	0.23	0.16	0.67
Pseudacanthotermes spiniger	0.17	0.01	0.36
	Soil-feeding termites		
Crenetermes albotarsalis	0.05	0.02	0.93
Cubitermes fungifaber	0.56	0.21	0.48
Cubitermes speciosus	0.02 ± 0.01	0.01 ± 0.01	0.85
Noditermes sp.	0.03	0.05	0.64
Procubitermes sp.	0.05	0.03	0.39
Thoracotermes macrothorax	0.07	0.01	1.09

*Assayed as described (7) for live termites, except where indicated. Mean values of duplicate analyses are reported for $n = 3$ to 5. †Determined (6) by measuring $^{14}CO_2$ reduction to $^{14}CH_4$ by gut homogenates in the presence of exogenously supplied H_2.

liters of CH_4 per day (*21*). However, the apparent ability of CO_2-reducing acetogens to outprocess methanogens for H_2 in the guts of wood- and grass-feeding termites [and in certain other habitats, including the colon of some humans (*22*)] is enigmatic. Thermodynamic and kinetic considerations suggest that CO_2 reduction to CH_4 (not acetate) is more likely to be the dominant H_2-consuming process (*15, 23, 24*). Clearly, other factors must affect competition for H_2 between acetogens and methanogens in habitats such as the termite gut. On the basis of this study, one additional factor appears to be the feeding guild of the host. However, we do not yet know whether it is the nature of the food consumed or other features accompanying evolution into a particular feeding guild (for example, modified gut anatomy or digestive physiology) that affect terminal H_2 and CO_2 processing by the resident microflora.

We have recently isolated in pure culture three strains of CO_2-reducing acetogenic bacteria, one from the gut of a higher and one from the gut of a lower wood-feeding termite, and one from the gut of a higher soil-feeding termite (*23, 25*). Each is a novel and different bacterial species, but, like other CO_2-reducing acetogens, none is strictly dependent on the presence of H_2 + CO_2. Each can ferment a variety of organic substrates for energy, including methoxylated aromatics, which are components of lignin. One of these isolates, *Sporomusa termitida*, has also been shown to be mixotrophic, that is, it can derive energy by simultaneous use of organic and inorganic (H_2 + CO_2) substrate mixtures (*26*). Mixotrophy may enhance the ability of acetogens to outcompete methanogens for CO_2 reduction in the guts of wood- and grass-feeding termites, particularly if organic substrates utilizable by acetogens are more readily available in termites from such feeding guilds.

The gradually increasing concentrations of CH_4 in the atmosphere, and its potential effect on global warming, have underscored our need to clarify the sources and sinks of this trace gas (*27*). Other investigators have suggested that termite emissions may be a significant source of atmospheric CH_4, with estimates ranging from less than 5% to more than 40% of the total annual global CH_4 production (*8–14*). However, we share with most of these investigators the belief that such estimates must still be viewed with caution, because of uncertainties in global estimates of termite numbers and activities and because the magnitude of CH_4 oxidation by soil bacteria in and around termite mounds may or may not be significant [see (*12–14*)]. Moreover, earlier estimates were made without information on rates of methanogenesis versus acetogenesis from H_2 + CO_2 for termites of different feeding guilds. It appears from the present study that, owing to the hydrogenotrophic activity of acetogenic hindgut bacteria, wood- and grass-feeding termites typically evolve less than 10% of the amount of CH_4 that might theoretically be formed. By contrast, fungus-growing and soil-feeding termites lack significant levels of bacterial acetogenesis from H_2 + CO_2 and are potentially more important sources of CH_4 emission.

Our findings are consistent with the observation by Zimmerman *et al.* (*11*) that a fungus-growing *Macrotermes* sp. and an unnamed species of soil-feeding termite displayed relatively high rates of CH_4 emission, but the specific values were not reported nor were they compared with rates of CO_2-reducing acetogenesis for those same specimens.

In any case, termites representing such feeding guilds are among the most abundant in many tropical ecosystems (*8–14, 28*) and should be important groups for more detailed study. As population estimates of specific termite feeding guilds become more reliable and as the specific origins of carbon for aceto- and methanogenesis by the gut flora become more defined, the data reported herein should help to clarify the contribution of termites and their gut microbes to atmospheric CH_4 production and to carbon and hydrogen flow through anoxic habitats.

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16. This technique alone cannot distinguish between H_2-dependent methanogenesis and methanogenesis in which CH_4 is produced from other potential substrates, for example, acetate. However, for termites examined so far methanogenesis has been shown to occur by H_2 reduction of CO_2 [and not by cleavage of acetate (*6*)], and H_2/CO_2 methanogenic bacteria have been isolated in pure culture from termite guts [J. Yang *et al.*, *Abstr. Annu. Meet. Am. Soc. Microbiol.* (1985), p. 160]. Moreover, exogenously added H_2 stimulates CH_4 emission from live termites up to twofold [A. C. Messer and M. J. Lee, *Microb. Ecol.* **18**, 275 (1989)]. Although the relative merits of "static" versus gas "flow-through" systems for measuring termite CH_4 emission have been discussed [see (*9, 10*)], we viewed our "static" system as a reasonable field estimate of H_2/CO_2 methanogenesis for the purpose of this study.
17. Experiments were done with freshly collected (Dansville, MI) or laboratory-maintained specimens of *R. flavipes*, or they were done within 48 hours of receipt of termites from the following sources: *C. formosanus* (Lake Charles, LA; provided by L. Williams, U.S. Department of Agriculture, Gulfport, MS); *C. cavifrons* (southern Florida; provided by S. L. Tamm, Marine Biological Laboratory, Woods Hole, MA); *P. simplex* (Coral Gables, FL; provided by G. Prestwich, State University of New York, Stonybrook); *P. occidentis*, *Amitermes* sp., *G. perplexus*, and *T. tenuirostris* (Santa Rita Range area, southwestern Arizona; collected with the help of W. Nutting, University of Arizona) *Z. angusticollis* and *N. costalis* (San Francisco Bay Park, CA, and forest near Frijoles, Panama, respectively; provided by J. Traniello, Boston University); and *N. nigriceps* (forest in Lesser Antilles; provided by B. L. Thorne, Harvard University). All specimens were from laboratory colonies, except for the Arizona termites which were freshly collected. The following species from the Republic of Congo were assayed shortly after collection by us from nests: *M. parvus* and *N. lujae* (forest near Brazzaville); *N. arborum*, *M. mulleri*, *C. albotarsalis*, *C. speciosus*, *Noditermes* sp., *Procubitermes* sp., and *T. macrothorax* (Mayombe rain forest); *T. rhodesiensis* and *C. fungifaber* (savannah near Niari); *P. militaris* (savannah near Brazzaville); and *P. spiniger* (sugar cane fields near Nkayi).
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Oligomerization of Ribonucleotides on Montmorillonite: Reaction of the 5′-Phosphorimidazolide of Adenosine

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The regiospecific formation of oligomers from unblocked monomers in aqueous solution is one of the central tenets in research on the origins of life on earth. Direct experimental support for this hypothesis has been obtained in studies of the condensation of the 5′-phosphorimidazolide of adenosine (ImpA) with itself and with P^1,P^2-diadenosine-5′,5′-pyrophosphate (AppA) in water in the presence of a montmorillonite clay. Oligomers of up to ten nucleotides in length are formed. Analysis of the trimers, tetramers, and pentamers formed from a 9:1 ImpA:AppA mixture has shown that 85% of the bonds formed are 3′,5′-linked and that any 2′,5′-linkages present are at the phosphodiester bond next to the 3′-terminus of the oligomers.

The observation of the catalytic role of RNA in processing RNA transcripts (*1*, *2*) suggests that RNA-like molecules (*3*) were the central biopolymers in the first life on earth. This postulate is strengthened by the observation of the catalytic elongation of a pentameric oligocytidylate [oligo(C)] by the ribozyme from a group I intron (*4*) and the efficient synthesis of the complementary oligoguanylate on tetrameric or longer oligo(C) templates (*5*). The relevance of these findings to the origins of life has always been tempered by the absence of a plausible prebiotic route to a 3′,5′-linked oligomer that would be amenable to elongation by ribozymes or template-directed replication (*6*, *7*). In previous research, mainly 2′,5′-linked oligoadenylates were formed from the phosphorimidazolide of adenosine (5′-ImpA). Reaction of 5′-ImpA in the presence of Zn^{2+} or Pb^{2+} resulted in the formation of tetrameric and pentameric 2′,5′-linked oligomers in yields of 25 and 35%, respectively (*8*, *9*). 2′,5′-Linked oligonucleotides, up to 16 units in length, are formed from the reaction of 5′-ImpA in the presence of UO_2^{2+} (*10*).

The conversion of monomers to dimers and trimers in the presence of Na^+-montmorillonite by reaction with a water-soluble carbodiimide [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC)] was reported previously (*7*). The condensation reactions of 5′-ImpA in the presence of Na^+-montmorillonite are reported here. Incubation of 1 ml of a 14.5 mM solution of 5′-ImpA in a solution (pH 8) of 0.2 M NaCl and 0.075 M $MgCl_2$ containing 50 mg of Na^+-Volclay (*11*) for 3 days at room temperature resulted in the reaction of all of the monomer and the formation of a 61% yield of oligomers. Analysis of the reaction products by high-performance liquid chromatography (HPLC) with an anion-exchange column (*12*) resulted in the detection of oligomers up to ten nucleotides in length (Fig. 1A). The absence of the P^1,P^2-diadenosine-5,5′-pyrophosphate (AppA), a reaction product of 5′-ImpA in the absence of Na^+-montmorillonite and the predominant product in the reaction of 5′-adenosine monophosphate in the presence of EDAC (*7*), suggested that the AppA formed was incorporated into the oligomeric products. This result was verified when a 9:1 mixture of 5′-ImpA:AppA gave less than a 1% recovery of AppA, as shown by reverse-phase HPLC analysis (*7*) when the reaction was performed under the same conditions. Anion-exchange HPLC (Fig. 1B) showed the formation of oligomers (67%) up to the decamer in length (*13*). Structural information on the reaction products from the 9:1 5′-ImpA:AppA reaction was obtained by collection of the respective peaks from the anion-exchange HPLC column and by the use of enzymatic and KOH hydrolyses to determine the structures of the oligomers in each fraction.

Hydrolysis with alkaline phosphatase (APH) was used to determine the percentage of each fraction with incorporated AppA groupings (*14*). The ratio of the area of the oligomer HPLC peak on ion-exchange chromatography after and before treatment with APH gave the proportion of oligomers with an incorporated AppA unit (Table 1). Comparison of retention times of the APH hydrolysis products with authentic samples of 3′,5′-A$(pA)_n$ made it possible to determine the amounts of 3′,5′-$(pA)_{n+1}$ (n = 2 and 3) in the trimer and tetramer fractions, respectively (Table 2). It followed from this determination that 2′,5′-ApA groupings are present only on the 3′-termini of the oligomeric chains and that the HPLC peaks with almost the same retention times as the 3′,5′-A$(pA)_n$ peaks were the corresponding $A^{3'}pA^{2'}pA$ and $A^{3'}pA^{3'}pA^{2'}A$ isomers in the trimer and tetramer fractions, respectively. The percentages of both isomers, obtained by HPLC analysis after treatment with APH, are given in Table 1.

Incubation of the separated fractions with ribonuclease T_2 (RNase T_2), an endonuclease that cleaves a 3′,5′-phosphodiester bond to the corresponding 5′-OH and 3′-phosphate (*15*), followed by analysis by anion-exchange chromatography and reverse-phase chromatography (*7*), resulted in the hydrolysis of the fraction and the detection of A, Ap, 2′,5′-ApA, pAp, pAppA, pAppAp, and $pAppA^{2'}pA$ (Table 1). Further hydrolysis of the RNase T_2 hydrolysate with APH resulted in the detection of A, AppA, 2′,5′-ApA, and

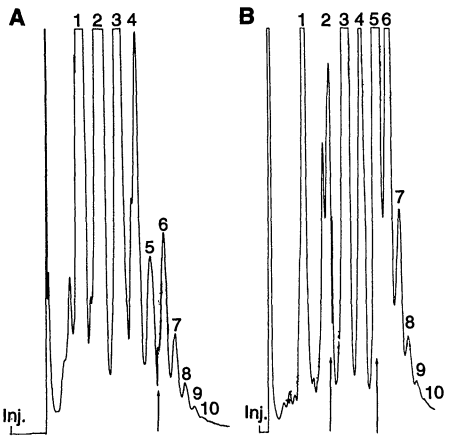

Fig. 1. (**A**) The anion-exchange HPLC separation of the oligomers formed from the reaction of ImpA on Na^+-montmorillonite. (**B**) The anion-exchange HPLC separation of the oligomers formed from the reaction of a 9:1 ImpA:AppA mixture on Na^+-montmorillonite. Arrows designate a change in recorder attenuation; Inj., sample injection. The numbers are the number of monomer units in the oligomers present in each peak.

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