BIRADICAL TETRAETHER LIPIDS FROM THERMOACIDOPHILIC ARCHAEBACTERIA

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INTRODUCTION

Archaebacteria are a novel class of micro-organisms, commonly encountered in exceptional ecological niches: methanogens in the absence of oxygen and in the presence of hydrogen and carbon dioxide, halophiles at high salt concentration, thermophiles at high temperature ranging from 60° to 105°. A variety of phylogenetic arguments has led some authors to propose archaebacteria as a third primary kingdom of living organisms, the two others being prokaryotes and eukaryotes, and to suggest that archaebacteria may have played a key role in the early history of life (for a review, see Woese, 1981).

From a chemical viewpoint, much is known about the structure of the molecular components of archaebacteria (see Kandler, 1982). Upon comparing archaebacteria with prokaryotes and eukaryotes, one is struck by the extensive chemical differences observed in the lipids, especially by contrast with the other components (nucleic acids, proteins, etc.) whose essential chemical features are preserved throughout all living organisms (Woese, 1981). These differences are sketched in Fig. 1. Sulfolobus solfataricus, whose lipids we have studied in this work, presents the extreme situation of all the lipid molecules consisting of two C_{40} ω - ω ' biphytanyl residues, with 0 to 4 cyclopentane rings per chain, ether linked at both ends to glycerol and nonitol (see Fig. 1; see also De Rosa et al., 1977, 1980, 1983).

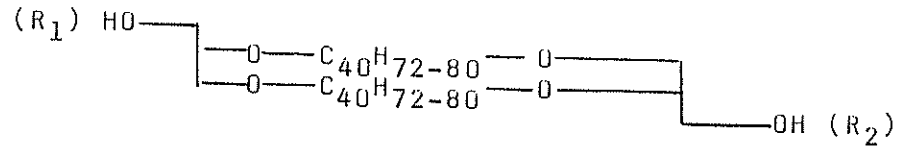
Presumably, and in spite of the difference in the ecological conditions, the membranes of archaebacteria, prokaryotes and eukaryotes, all carry out similar physiological functions. The question -- which bears on the general problem of the physiological role of lipids -- may thus be asked how so widely different molecules manage to perform the same functions and why is that variability required for life.

We summarize in this paper the physico-chemical aspects of a recent study of the polymorphism of lipid extracts from S. solfataricus (Gulik et al., 1985); the biological implications of that work are discussed by Luzzati & Gulik (1985).

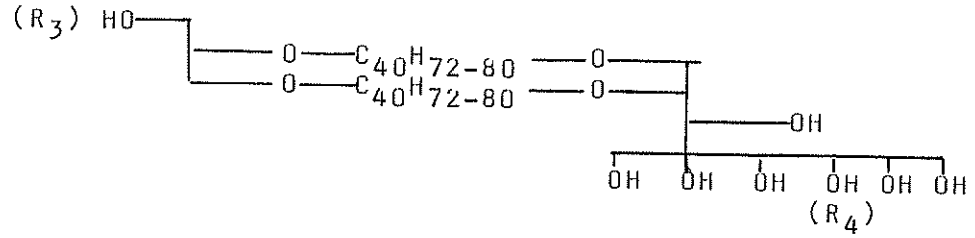
MATERIAL AND METHODS

The lipids were extracted from S. solfataricus, strain MT-4, grown at 87°C, pH 3.5 (De Rosa et al., 1975). All the lipid molecules are derived from two compounds:

I - glycerol dialkyl glycerol tetraether (GDGT)



II - glycerol dialkyl nonitol tetraether (GDNT)



where ($-C_{40}H_{72-80}-$) represents the biphytanyl chains. In the "native" lipid extract most of the hydroxyl hydrogen atoms are substituted by a variety of polar groups (R_1 to R_4); it is worth noting, though, that approximately 20% of the lipid headgroups are unsubstituted glycerols (namely R_1=H or R_3=H, see Gulik et al., 1985).

We summarize below the results obtained with three lipid preparations: the hydrolytic fractions GTGT and GTNT (see above), and their polar lipid extract (PLE). These lipids are remarkably stable: chromatographic analyses carried out on samples that had undergone the harshest thermal treatments involved in the X-ray scattering study failed to detect any chemical alteration.

The X-ray scattering experiments were performed at different temperatures on samples containing controlled amounts of lipid and water. Unless otherwise stated, the observations reported below are all considered to be at thermodynamic equilibrium, on the ground that the experimental results are independent of the previous thermal treatment of the sample. As usual in crystallographic studies of lipids, the short-range conformation of the hydrocarbon chains can be distinguished operationally from the long-range organization of the lipid molecules.

As in other lipids, the hydrocarbon chains of bipolar lipids adopt two main types of short-range conformations. One (type α), predominant at high temperature, is highly disordered (Luzzati, 1968; Luzzati & Tardieu, 1974). The other (type β'), predominant at low temperature, corresponds to stiff chains, all parallel to each other and organized with rotational disorder according to a two-dimensional lattice (Luzzati & Tardieu, 1974). Apparently, the presence of side methyl groups and of isopentane cycles is compatible with this conformation, as is the presence of double bonds in fatty acid chains.

EXPERIMENTAL RESULTS

3a - Glycerol diakyl glycerol tetraether (GDGT)

The phase diagram of GDGT is quite simple: it contains only one ordered phase with stiff chains, $L\beta'$, which takes up a very small amount of water (see

Fig. 3). The fact is noteworthy that in GDGT (and not in the other lipids studied in this work) the long-range periodic order collapses as the short-range conformation of the hydrocarbon chains becomes disordered. The absence of liquid-crystalline phases, and also the very small degree of hydration, indicate that the glycerol headgroups of GDGT do not share the polar nature of the headgroups of amphiphilic lipids and segregate preferentially in the disordered hydrocarbon matrix, rather than at the polar/apolar interface.

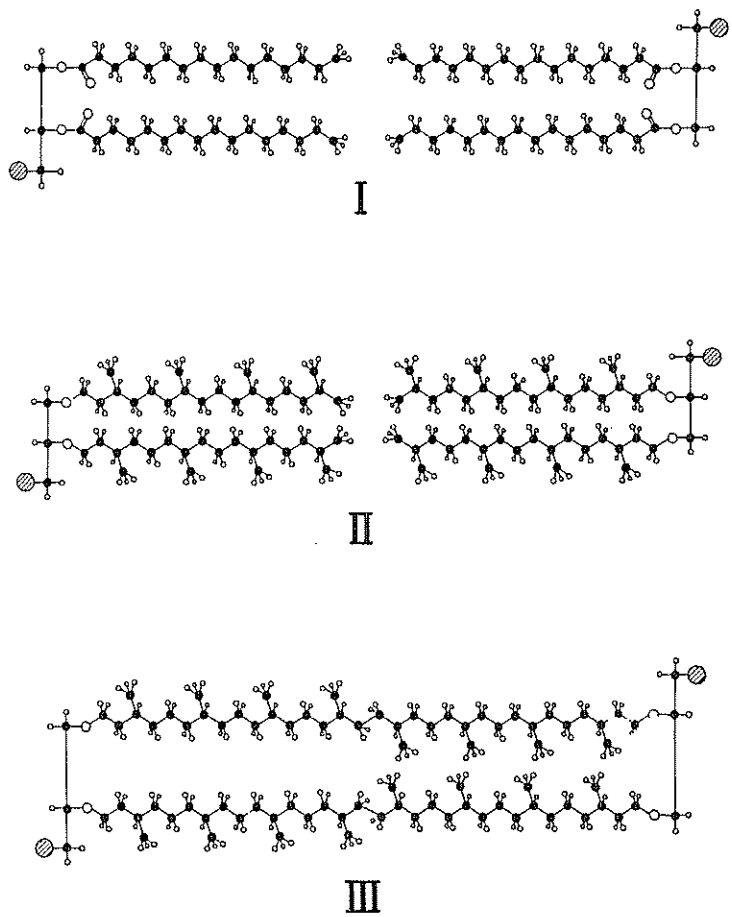


Figure 1

Three examples of lipid molecules apposed as in lipid bilayers. Filled circles, carbon atoms; open circles, oxygen atoms; small circles, hydrogen atoms; hatched circles, polar residues.
I. lipid of eukaryotes and prokaryotes: the hydrocarbon chains are linear (palmitate in this case) and ester linked to glycerol. II and III. lipids of archaebacteria: the hydrocarbon chains are branched (isopranyl) and ether linked to glycerol. Each molecule of type II contains 2 C_{20} chains linked to glycerol; lipids of this type are commonly found in halophiles and in methanogens. III is a dimer of II: it consists of 2 C_{20} chains ether lined at both ends to glycerol groups. The lipids of S. solfataricus studied in this work belong to type III. with 0 to 4 cyclopentane groups along each of the isopranyl chains (from Gulik et al., 1985).

3b - Glycerol diakyl nonitol tetraether (GDNT)

The portion of the phase diagram explored in this work is shown in Fig. 2. Three phases were studied: P, Q^{230} and H. The structure of the phases P and H is represented in Fig. 3; the structure of the phase Q^{230} is similar to that of the same phase of the polar lipids, discussed below and represented in Fig. 4.

An inspection of the structure of the phase P and, in the case of the phases H and Q^{230}, an analysis of the evolution of the dimensions of the structure elements with varying hydration and temperature (Gulik et al., 1985), confirm the conclusion reached in the study of GDGT (see above) that the unsubstituted glycerol headgroups partition preferentially in the hydrocarbon regions rather than mixing with the nonitol headgroups at the polar/apolar interfaces.

3c - Polar lipid extract (PLE)

The phase diagram of PLE contains a variety of phases (see Fig. 2), much like the phase diagrams of lipid extracts from other organisms. Several features, some of which are strikingly different with respect to other lipids, are worth pointing out.

As in GDGT and GDNT, the unsubstituted glycerol headgroups are distributed preferentially in the hydrocarbon regions, rather than mixed with the other headgroups at the polar/apolar interfaces.

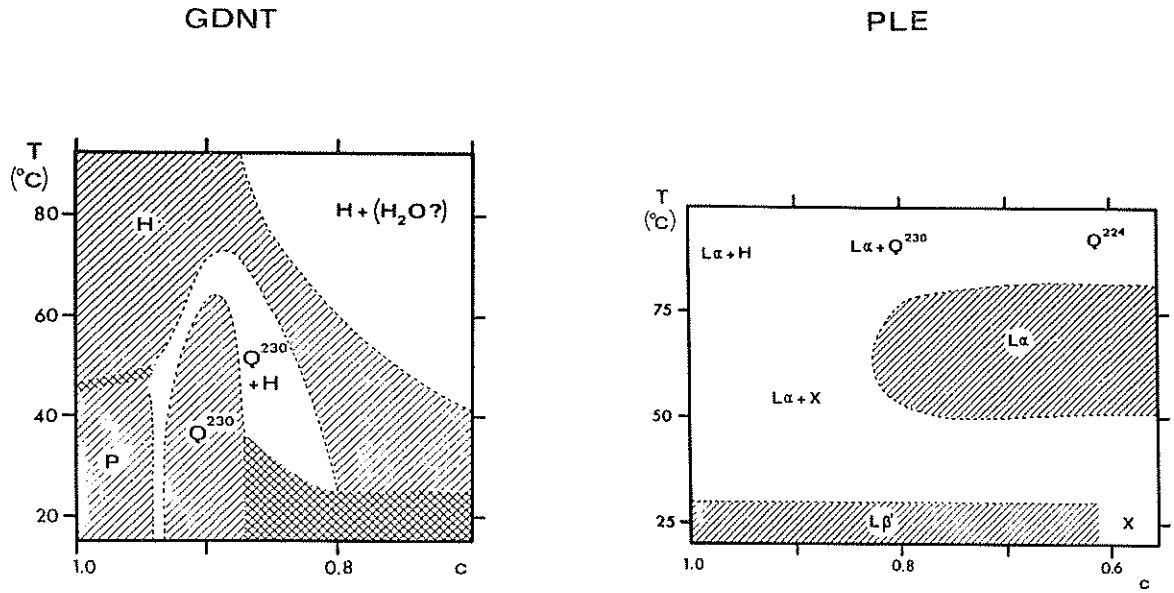


Figure 2

Phase diagram of the system GDNT-water and PLE- water. The one-phase regions are hatched; the position of the phase boundaries was not determined with great accuracy. A question mark indicates the presence of some additional disordered phase. X shows the presence of unidentified sharp reflections. The conformation of the chains is disordered in all the phases, with the exception of the cross-hatched area in GDNT and of the phase Lα' of PLE.

The hexagonal phase H is observed over a narrow region of the phase diagram, at high temperature and low water content (see Fig. 2). Its structure is similar to that of the phase H of GDNT (see Fig. 3).

The lamellar phase $L\alpha$ displays some unusual features (Gulik et al., 1985). This phase is observed over an extended range of the phase diagram: the thickness - and presumably the structure - of the lipid lamellae is found to be independent of the water content. Most surprisingly, though, the average thickness of the hydrocarbon layer is larger than the fully extended length of the hydrocarbon chains, rather than being substantially smaller as in the $L\alpha$ phase of other lipids. As a consequence, whenever a molecule spans the lipid membrane, the local thickness is smaller than average: some thickening elsewhere must compensate for that thinning. Two types of structures can be proposed to explain

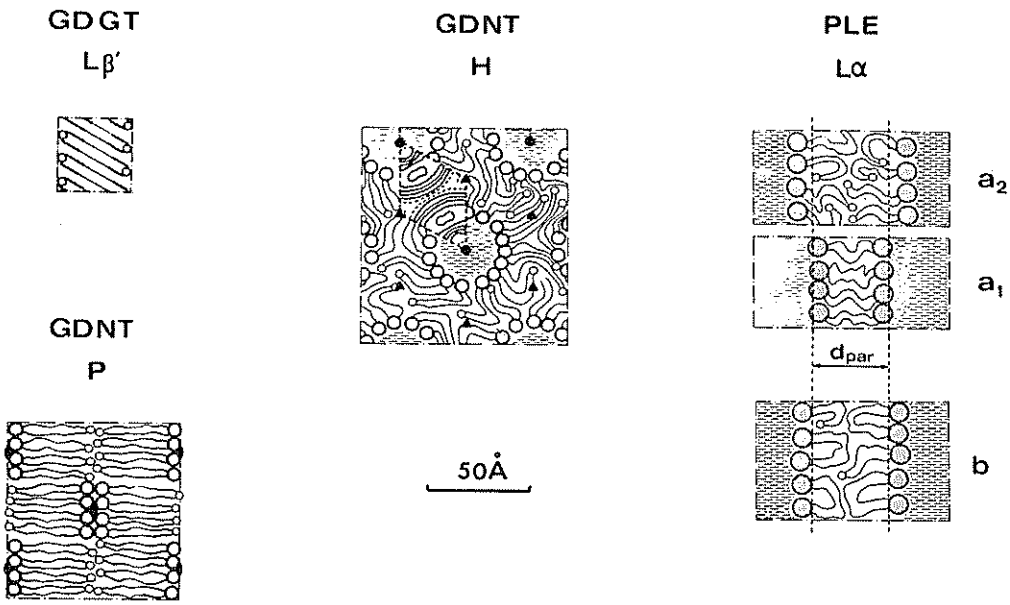
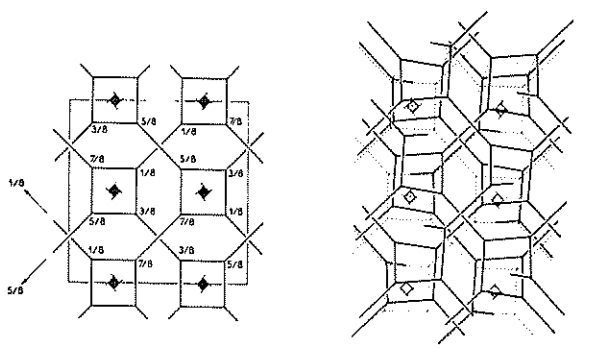


Figure 3

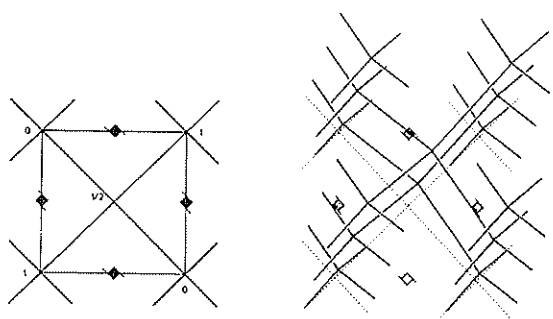
Schematic representation of the structure of some of the phases (see Fig. 1). Small circles: unsubstituted glycerol headgroups. Large hatched circles: substituted glycerol, substituted and unsubstituted nonitol headgroups. Wriggles: hydrocarbon chains in the disordered (α) conformation. Straight lines: stiff chains in the β' conformation; in the case of the phases L ' of GDGT and P of GDNT the lighter lines represent the segments of the chains devoid of cyclopentanes (see Gulik et al., 1985).
GDGT, phase Lβ'. Section of one lipid lamella. The hydrocarbon chains are stiff and parallel, and are tilted with respect to the plane of the lamella (type β').
GDNT, phase P. The structure consists of lipid lamellae, in each of which the glycerol headgroups segregate away from the nonitol headgroups. The structure is organized according to a two-dimensional centered rectangular lattice. The figure represents one section of the structure.
GDNT, phase H. The structure consists of rods filled by water and by the polar groups of the lipid molecules, and covered by the nonitol headgroups. The glycerol headgroups segregate away from the polar regions. The lattice is two-dimensional hexagonal. The figure represents a section of the structure, perpendicular to the 6-fold axis. The insert shows the electron density distribution.
PLE, phase Lα. (See text.)

this observation: **1)** The phase contains a heterogeneous mixture of two types of lamellar domains: one domain (a_1 in Fig. 3) consists of a monolayer of bipolar molecules (those whose headgroups are substituted glycerol and nonitol) spanning the lipid layer; the other domain (a_2 in Fig. 3) is a bilayer of the other class of molecules, with the unsubstituted glycerol headgroups embedded in the hydrocarbon matrix. The lamellar structure could be a statistical alternation of the two kinds of uniform lamellae (one entirely of type a_1, the other of type a_2), or else an ordered stacking of heterogeneous lamellae, each being a patchwork of the two types of domains. **2)** All the lamellae are smooth and homogeneous. The only way to avoid thickness fluctuations is to assume that all the bipolar lipid molecules are U-shaped, with the two headgroups located on the same side of the lamella (b in Fig. 3).

Several observations are in favour of the structure of type 1: the presence of diffuse X-ray scattering at small angles (Gulik et al., 1985) the absence of a conspicuous electron density trough in the middle of the hydrocarbon region (see Fig. 4. in Gulik et al., 1985), the absence of a lipid fracture in freeze etching electron micrographs (Weiss, 1974). Moreover, recent observations show that one



Q^{230}



Q^{224}

Figure 4

The structure of the cubic phases. The thick lines represent the axes of the rods. The two structures consist of two three-dimensional continuous networks of polar rods, mutually intertwined and unconnected. Left frames: representation of the unit cell with the position of the axes of the rods and of some of the symmetry elements. Right frames: perspective view of the structure. Upper frames: phase Q^{230}. Note that the rods are linked coplanarly three by three. Lower frames: phase Q^{224}. Note that the rods are linked tetrahedrally four by four (from Gulik et al., 1985).

particular lipid fraction from PLE containing bipolar molecules (namely with R_1 to R_4 different from H, see above) form a lamellar phase with lipid molecules spanning the hydrocarbon layer (like a_1 in Fig. 3), whereas another fraction consisting of molecules with one unsubstituted glycerol headgroup yields more complex phases in which the glycerol headgroup partitions preferentially in the hydrocarbon region.

The two cubic phases, Q^{224} and Q^{230}, have been observed and described in several other lipid-water systems. Their structures, represented in Fig. 4, consist of two intertwined networks of rods: the interior of the rods is filled by the polar moiety of the systems (as depicted for the phase H of GDNT in Fig. 3). The novelty of S. solfataricus lipids is that the two cubic phases display a remarkable degree of metastability. Whenever a lipid-water sample adopts either cubic phase (for example in a heating scan), that structure sets in almost irreversibly; if the sample is subsequently brought back and kept at a lower temperature it may take weeks before the cubic phase gives way to the phase that was observed at that temperature in the initial heating scan. Eventually, though, the non-cubic phase is recovered and the thermal cycle can be scanned again. We discuss below this phenomenon.

With regard to the phase(s) with stiff chains, little effort was invested in analyzing their structure, with the exception of the phase $L\beta'$ of GDGT (see above and Fig. 3).

COMMENTS AND DISCUSSION

As in other lipid-water systems, whenever the chains are stiff the structure is lamellar. Melting of the chains gives way to a variety of phases of widely different structures. This type of polymorphism, bringing together long-range crystalline order and short-range liquid-like disorder is a characteristic property of lipids, not shared to nearly the same extent by any other family of chemical compounds.

The presence of unsubstituted glycerols is exceptional for lipids of biological origin: mono and diglycerides are indeed virtually absent in the membranes of prokaryotes and eukaryotes, as are phytanyl glycerides among diether isopranyl lipids of archaebacteria (Kates & Kushwaha, 1978; Kushwaha et al., 1982). Moreover, the unsubstituted glycerols do not share the polar character of the other headgroups and partition preferentially in the hydrocarbon matrix, rather than at the polar/apolar interface. Consequently, a substantial fraction of S. solfataricus lipids behave as amphiphilic molecules twice as long as ordinary lipid molecules and are thus capable of spanning a hydrocarbon layer twice as thick as that of ordinary membranes (namely 70 Å instead of 35 Å). The presence in the lipid matrix of these unusual molecules brings additional flexibility to the lipid-water phases: an uneven distribution of the unsubstituted glycerols is indeed involved in the absence of a proper lamellar phase and in the structure of the cubic phases (see Fig. 3 and Gulik et al., 1985).

The fact is noteworthy that the phases stable under conditions close to "physiological" - high water content and high temperature - are the cubic ones. Indeed, the rule inferred from previous studies of other lipids was that only the lamellar phase $L\alpha$ is observed at physico-chemical conditions close to those which prevail in the living organism from which the lipids are extracted; this rule, moreover, has often been invoked to dispose of the non-lamellar lipid phases as crystallographic oddities devoid of biological significance. This argument, whatever its relevance, clearly is pointless in the case of S. solfataricus lipids. The whole body of experimental observations corroborates an early suggestion (Luzzati et al., 1968) that from a heuristic viewpoint, a loose fabric of rod-like lipid elements offers a more flexible and attractive model for biological membranes than the inert bilayer. Luzzati & Gulik (1985) further develop this point and suggest, on the

basis of a remarkable epitaxial relationship of the Q^{224} lipid phase with the protein envelope of the cells, that the membrane may perhaps adopt an unconventional type of structure, displaying unusual fractal properties.

The metastability observed in the phase transitions involving the cubic phases is another extraordinary property of S. solfataricus lipids: in lipids, all the transitions between phases *with disordered chains* have been reported to be fast, at least at the scale of X-ray scattering experiments (typically one hour). This phenomenon can be explained by the presence in S. solfataricus lipids of a high fraction of molecules with two polar headgroups, and by the structure of the cubic phases. The polar headgroups are indeed anchored on surfaces belonging to the two unconnected networks of rods (see Fig. 3); besides, many of the molecules have their two headgroups attached each to one of the two networks. Under these conditions, the migration of the molecules is limited by the diffusion of the two headgroups and by the entanglements of the chains: any phase transition involving long-range diffusion of the lipid molecules is likely to be slow. Clearly, this phenomenon does not affect lipid molecules with only one polar headgroup. This phenomenon may have interesting biological implications, related to the unusual habitat of thermoacidophilic bacteria (Gulik et al., 1985; Luzzati & Gulik, 1985).

Another interesting observation is that at high temperature, the phase present at high water content is the cubic Q^{224} (see also Hyde et al., 1984). It may thus be presumed that the "liposomes" of the S. solfataricus lipids consist, at high temperature, of lumps of the phase Q^{224} floating in excess water; according to the phase diagram these lumps may be expected to give way, at lower temperature, to some other phase (probably lamellar). This type of liposome can be visualized as a sponge-like object highly penetrable to water -- the diameter of the polar channels can be larger than 80 Å (see Table 2 of Gulik et al., 1985) -- with an extended and convoluted polar/apolar interface. This structure is remarkably different from that of ordinary liposomes, which usually take the form of onion-like multilamellar particles highly impenetrable to the external aqueous medium. These features--extended interfacial area (nearly 500 m^2/g), easy penetration of water, reversible temperature-induced structure transitions, and also the strict chiral specificity of the lipid molecules (De Rosa et al., 1983) -- potentially invest S. solfataricus liposomes with interesting catalytic properties (see Luzzati & Gulik, 1985).

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