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The molecular structure of hop latent viroid (HLV), a new viroid occurring worldwide in hops

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ABSTRACT

A new viroid which does not seem to produce any symptoms of disease, and is therefore tentatively named hop latent viroid (HLV) was found to occur worldwide in hops. HLV proved to be infectious when mechanically inoculated onto viroid- and virus-free hops. The viroid nature of HLV was also substantiated by sequence analysis which revealed that HLV is a circular RNA consisting of 256 nucleotides, that can be arranged into the viroid-specific, rod-like secondary structure. HLV also contains the central conserved region typical for most of the presently known viroids. However HLV does not contain the viroid-specific oligo(A) stretch in the upper left part of its rod-like molecule. Because of this feature and a sequence similarity with the prototypes of the other viroid groups below 55%, HLV can be regarded as the first member of a new viroid group.

INTRODUCTION

To evaluate the status of the possible viroid infection of German hops we have made a survey in the main German hop growing region "Hallertau" which is located 80 km north of Munich. With 17.000 hectares of hop gardens it is the largest hop producing region of the world. We detected a new viroid in practically all of the hop cultivars tested. Since this new agent does not seem to produce any symptoms of disease in hops, it is tentatively named hop latent viroid (HLV). When we extended our survey to the other main hop growing areas of the world (to be published in detail elsewhere) we found that HLV occurs worldwide in most of the hop cultivars tested so far. We also found that the wellknown hop stunt viroid (HSV) (1) is, indeed, only present in Japanese hops as previously reported (2-5), and that such HSV-infected hop plants may contain HLV in addition.

Here we report on the characterization of HLV, on its

primary and secondary structure and on the similarity of its sequence with the one of the other viroids presently known.

MATERIALS AND METHODS

Plants and bioassays

The hop (*Humulus lupulus*) cultivars (cvs) surveyed in our study were grown in hop gardens in the field. All infectivity assays were performed in the greenhouse using the commonly used viroid host plants cucumber (*Cucumis sativus*), tomato (cvs "Rutgers" and "Rentita"), *Chrysanthemum morifolium* (cvs "Mistletoe" and "Bonnie Jean", *Benincasa hispida*, *Gynura aurantiaca*, *Nicotiana glutinosa* and a virus- and viroid-free clone of the hop cv "Hersbrucker". The absence of hop mosaic virus and prunus necrotic ringspot virus in this clone material was ascertained by ELISA tests, whereas the absence of viroids was ascertained by "return electrophoresis" and by Northern blot hybridizations with various viroid-specific probes. In the case of tomato, *Chrysanthemum* and *N. glutinosa*, mechanical inoculation was done by rubbing the inoculum into the leaf in the presence of carborundum. Cucumber, *Benincasa*, *Gynura* and the hop plants were inoculated by puncturing about 15-20 times with a pointed scalpel their stem through the inoculum applied to it. For inoculation 25 ug of the HLV-containing 2M LiCl-soluble RNA isolated from HLV-infected hop cones were used per plant. For bioassaying the cloned HLV, 2 ug/plant of the *in vitro* synthesized dimeric HLV(+)RNA were applied. The replication and accumulation of HLV in the inoculated plants was ascertained by molecular hybridization analysis.

Preparation of viroid containing RNA samples

For the detection of possible viroid infections in hops and for viroid purification total nucleic acid was isolated from hop tissue with phenol. Source materials were young hop leaves, young hop cones and mature cones that had been commercially dried at about 60-65^{0}C or air-dried at room temperature. For nucleic acid extraction the hop tissue was homogenized in the precence of TEMS buffer (0.1 M Tris-HCl, 0.1 M NaCl, 0.01 M EDTA, 0.01% 2-mercaptoethanol, pH 7.5) and extraction phenol (1 kg phenol, 1 g 8-hydroxychinoline, 250 ml chloroform, 300 ml

TEMS). Per gram fresh tissue 10 ml buffer and 10 ml phenol were used whereas per gram dried tissue 50 ml buffer and 50 ml phenol were needed to allow thorough homogenization. The homogenate was centrifuged at 20.000 rpm, the resulting aqueous phase was recovered and rehomogenized with an equal volume of extraction phenol. Upon centrifugation the total nucleic acids present in the aqueous phase were precipitated from it with 2.5 volumes of ethanol, pelleted by centrifugation and dried in the vacuum. The dried nucleic acid pellets were then extracted with 2M LiCl in TEMS buffer. The resulting 2M LiCl-soluble RNA (containing the viroid RNA together with the cellular 7S RNA, 5S RNA and tRNA) was used for electrophoretic analysis and for further purification of the viroid RNA by HPLC.

Viroid detection by "return"-electrophoresis

For the detection of viroids by bidirectional electrophoresis, the so-called "return"-electrophoresis of Schumacher *et al.* (6) was applied. In place of vertical 140x100x1.5 mm slab gels cast between glas plates, 0.5 mm thick 5% horizontal polyacrylamide slab gels were used which had been cast on precoated 260x203 mm plastic sheets (Gel Bond PAG-Film, LKB product 1850-211). Electrophoresis was carried out in a Multiphor II (LKB) apparatus, and samples containing 5 ug of 2M LiCl-souble RNA were separated per lane.

Viroid detection by molecular hybridization

To detect the presence of viroids in the nucleic acid preparations from hops, molecular hybridization of dot spots and Northern blots was performed as previously described (7). As probes for viroid detection radioactively labeled dimeric HSV(-)RNA and dimeric HLV(-)RNA were synthesized *in vitro* using T7-induced RNA polymerase (8) and cloned dimeric HSV- and HLV-cDNA (9) as template. For Northern blot analysis 1 ug of the 2M LiCl-soluble RNA fraction was separated per lane, whereas 5 ug of the same fraction were used for dot spotting. For testing large numbers of samples also crude buffer extracts were used. They were prepared from dried cones, and dot spotted according to the method of Owens and Diener (10). In this case an aliquot equivalent to 10 mg of dried hop tissue was dotted. In Northern blots HSV and HLV can be detected simultaneously as two

distinct bands when hybridization is carried out with HSV-specific RNA at 55^{0}C. Both viroids can then be discriminated by "washing" the blots for 16 hours at 75^{0}C in a solution of 2xSSC containing 0.1% SDS, in which case the HSV-specific signal remains whereas the HLV-specific signal disappears. When the HLV probe is used for detection and discrimination, the signal response is vice versa.

Purification of HLV by HPLC

For sequence analysis HLV was partially purified by HPLC using the 2M LiCl-soluble RNA from 250 g of freshly harvested young cones of a single symptom-free hop plant of the cultivars "Record", "Progress" and "Urozani", respectively. To purify the viroids from the Japanese hop cultivar "Kirin", freeze-dried leaf material was used which had been kindly provided by Dr. Shikata, Sapporo, Japan. HPLC was performed on a Nucleogene-500-DEAE-7 column (Diagen, Düsseldorf, FRG) as previously described (11) but 30 mM Na-phosphate pH 6.5 containing 50 % formamide was used as buffer. The RNA was eluted from the column by a linear gradient of 0-1.2 M NaCl in this buffer. HLV was detected in the eluted fractions by dot spotting aliquots on a Gene Screen Plus membrane, and subsequent molecular hybridization at 75^{0}C, using an *in vitro* synthesized HLV-specific RNA as probe. The HLV-containing ethanol-precipitated fractions were desalted by column-chromatography on BioGel A-0.5m and pooled for sequence analysis.

The yields of HLV were approximately 10 ug of RNA/100 g of fresh cones. It is noteworthy that depending on their age and on the season, the HLV concentration in hop leaves can be 10-1000 times lower than in the cones of the same plant, which may preclude reliable viroid detection in leaf tissue.

Sequence Analysis of HLV

End-labeling of viroid-specific DNA oligonucleotides and reverse transcription of HLV RNA with M-MuLV reverse transcriptase (BRL) were done as previously described (12). For this purpose the following four synthetic oligonucleotides were used as primers: first the "universal primer" for PSTV-like viroids pPSTV 16 (5'-dCTCCAGGTTT CCCCGG-3'), and later the three HLV-specific primers pHLV 20 (5'-dCGAACAAGAA GAAGCCGAAG-

3'), pHLV 25 (5'-dTCCCCGGGGA TCCCTCTTCG AGCCC-3') and pHLV 28 (5'-dCCACCGGGTA GTTTCCAACT CCGGCTGG-3'). The corresponding primer-extended HLV-cDNAs were sequenced by a combination of the five G-, G/A-, T/C-, C- and A/C-specific chemical cleveage reactions according to Maxam and Gilbert (13) and the T-specific reaction according to Rubin and Schmid (14) so that possible sequence heterogeneities also within purines and pyrimidines could be detected.

Computer calculations

The secondary structure of HLV was calculated on a VAX computer type MicroA (Digital) with the RNA-folding-algorithm of Jacobsen and Zucker (15). The free energy values of the structural parameters were those described by Freier *et al.* (16).

The sequence similarity between HLV and the other sequenced prototype viroids was determined with the UWGCG sequence analysis program GAP, version 5.0 June 1987, using a gap-weight of 5 and a gap-length-weight of 0.

RESULTS

Detection and partial characterization of HLV

The 2M LiCl-soluble nucleic acid fractions isolated from the leaves and cones of symptomless, apparently healthy plants of six different hop cultivars (cvs) grown in the "Hallertau" contained a single circular RNA as demonstrated by "return-electrophoresis" (Fig. 1, lane 2-6 and 8). For identification purposes, this RNA was termed hop latent viroid (HLV). Lanes 7 and 9 of Fig. 1 show that HLV was not present in plants of the two cvs "Hersbrucker" and "Spalter". For comparison, the nucleic acid extracts from tomato leaves infected with the previously described HSV were co-electrophoresed (Fig. 1, lanes 1 and 10). Although return-electrophoresis does not allow an exact size determination, the comparison clearly shows that in this gel system HLV and HSV migrate differently which indicated that they also differ in size.

The difference between HLV and HSV became fully evident when the nucleic acid extracts from representative hop cultivars from various hop growing areas of the world were analyzed

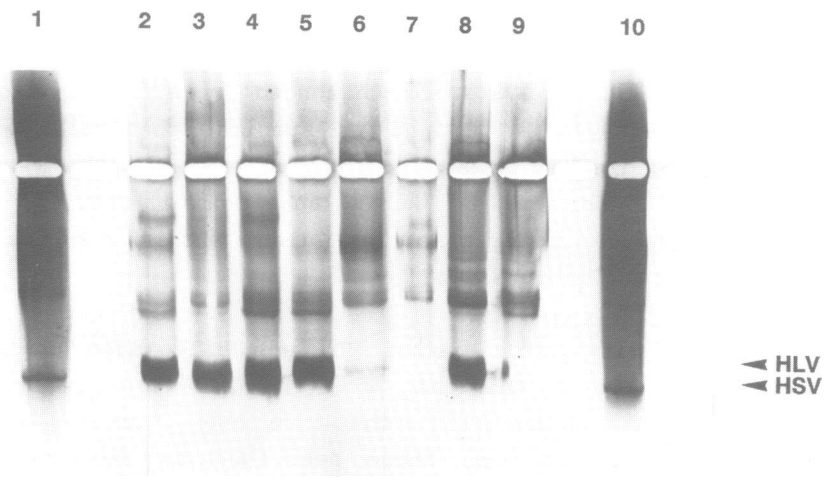
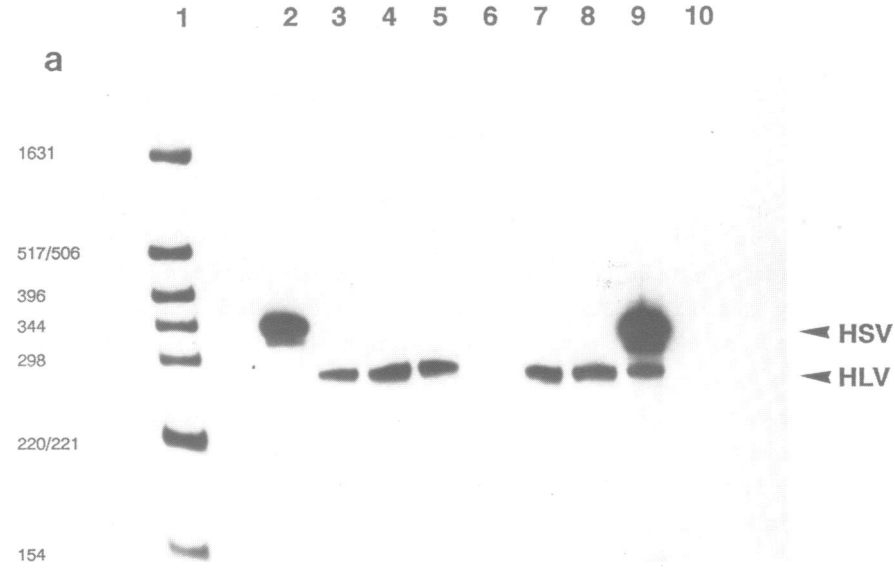


Figure 1. Silver-stained 5% polyacrylamide gel from a typical screening experiment in which 2M LiCl-soluble RNA samples from leaf tissue of the HLV-infected hop cultivars "Hallertauer", "Record", "Northern Brewer", "Brewers Gold", "Progress" and "Perle" (lanes 2-6 and 8), of the two viroid-free plants of the cvs "Hersbrucker" and "Spalter" (lanes 7 and 9) all grown in the "Hallertau" and of HSV-infected tomato leaf tissue (lanes 1, 10) had been separated by "return-electrophoresis". The band of the circular RNA of HLV and HSV is indicated by an arrowhead.



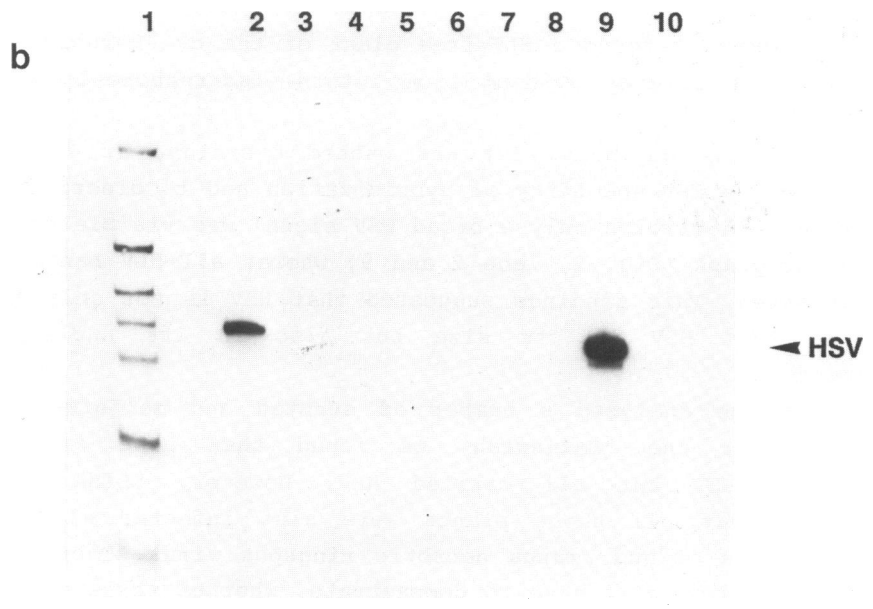


Figure 2. Autoradiographs after molecular hybridization with HSV-specific RNA transcripts of a Northern blot from a 5% polyacrylamid gel on which samples of individual plants of the following hop cultivars had been separated: "Cascade" from Oregon, USA (lane 3), "Wye Challenger" from Kent, England (lane 4), "Record" and "Hersbrucker" from the "Hallertau" (lane 5 and 6), "Aurora" from Yugoslawia (lane 7), "Dunar" from Hungary (lane 8) and "Kirin" from Japan (lane 9). Leaf RNA from the HSV-infected cucurbit *Benincasa hispida* (lane 2), from a viroid-free plant of the German cultivar "Spalter" (lane 10) and radioactively 5'-endlabelled Hinf I fragments of pBR 322 DNA (lane 1) were used as size marker.
a: Autoradiograph after hybridization at low stringency (55^0C). Under this conditions the HSV-complementary probe hybridizise not only with HSV but also with the smaller HLV as indicated by arrow heads. It is noteworthy that the Japanese hop cultivar contains HSV and HLV.
b: Autoradiograph of the Northern blot in Fig. 2a after it had been washed overnight at 75^0C to increase the stringency of hybridization. Under these conditions all HLV signals disappeared, and only the broad HSV band remained.

in Northern blots using a probe complementary to the authentic HSV. Molecular hybridization under low stringency (55^0C) demonstrated (Fig 2a) that in Japanese hops (lane 9) two signal bands appear, one of which is evidently caused by HSV (lane 2) whereas the faster migrating second band co-migrates with HLV

as the only viroid present in non-Japanese cvs (lanes 3, 4, 5, 7, 8). Again we found a HLV-free plant of the cv "Hersbrucker" (lane 6) as already evident from return-electrophoresis (Fig. 1, lane 7).

When the Northern blot was washed overnight at 75^0C to increase the HSV-specifity of hybridization and to discriminate between both viroids only a broad HSV signal was visible on the autoradiograph (Fig. 2, lane 2 and 9) wheras all HLV bands had disappeared. This findings suggested that HLV is not only different from HSV in its size but also in its nucleotide sequence.

When we analyzed a number of stunted and malformed hop plants from the "Hallertau" we found that none of them contained HSV but all carried HLV. However, ELISA tests revealed that all these plants were also infected with hop mosaic virus or/and prunus necrotic ringspot virus. Therefore, further studies will have to demonstrate, whether these disease symptoms are induced by certain combinations of these viruses with HLV or whether they are caused by a pathogenic sequence variant of HLV. It should also be noted that in different hop gardens individual viroid-free plants can be found amongst the HLV-infected hop cvs.

The complete nucleotide sequence of HLV

The complete sequence of HLV was established at the level of its cDNA which was synthesized by reverse transcription using the HPLC-purified HLV RNA of the German cultivar "Record". Since molecular hybridization had shown that HLV shares certain sequence elements with HSV and potato spindle tuber viroid (PSTV) (17), in pilot experiments a set of HSV- and PSTV-specific DNA primers were tested for their capacity to prime HLV cDNA synthesis. The "universal" PSTV-specific pPSTV 16 which is complementary to a sequence motif in the upper part of the central conserved region of most of the presently known viroids allowed us to synthesize a HLV-specific DNA which was sequenced according to the method of Maxam and Gilbert (13). The sequence information obtained in this way was used to manufacture the HLV-specific primer pHLV 20. The sequence data obtained with this primer led to the synthesis of the two addi-

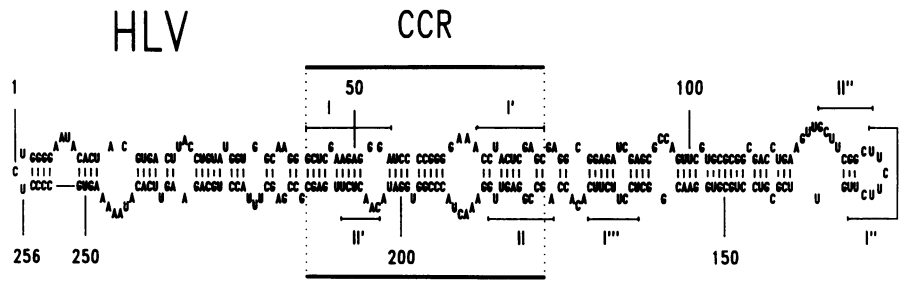


Figure 3. The sequence of HLV as arranged in a thermodynamically optimized secondary structure. The numbering of the residues follows the convention established for PSTV (17). CCR: central conserved region; the sequence motifs I and II are partly complementary to the motifs I', I'', I''' and II'and II'', respectively.

tional primers pHLV 25 and pHLV 28. By elongating these four primers it was possible to synthesize and sequence four overlapping HLV cDNAs. From these data the complete nucleotide sequence of HLV and its secondary structure could be constructed which is presented in Fig. 3. It shows that the circular RNA of HLV consists of 256 nucleotides which can potentially form a rod-like structure with a high degree of base-pairing like all the other known viroids. In total 65.5% of the residues are base-paired and the base-paired residues consist of 63.1% G:C, 27.4% A:U and 9.5% G:U base pairs.

The HLV sequence was analyzed for putative translation products in both the plus and minus strands using AUG and GUG as the possible initation codons. One open reading frame (ORF) starting with AUG is found in the plus strand (residues 30-242) which shows that HLV could potentially encode a polypeptide of 71 amino acids (not including the termination codon). In the minus strand no ORF starting with AUG could be found. Six GUG initation codons exist on the plus and four on the minus strand of HLV RNA. The corresponding longest ORFs on the plus strand (residues 17-114) and minus strand (residues 86-232) could potentially encode for peptides consisting of 118 and 49 amino acids, respectively. The biological significance of these putative translation products is not known.

In order to search for possible sequence variants of HLV,

Table 1 Sequence similarities between the prototypes of the different viroid groups

Viroid	Compared with[a]	Sequence similarity (%)
HLV	PSTV	51
	HSV	45
	CCCV	54
	ASSV	46
	ASBV	36

a: PSTV: potato spindle tuber viroid; HSV: hop stunt viroid; CCCV: coconut cadang cadang viroid; ASSV: apple scar skin viroid; ASBV: avocado sunblotch viroid.

three additional viroid isolates were purified and sequenced: an HLV isolate from the German hop cv "Progress", an isolate from the Russian cv "Urozani", and an isolate from the Japanese cv "Kirin". It was found that the sequence of these three HLV isolates is identical to the HLV sequence presented in Fig. 3. The sequence analysis of additional HLV isolates is in progress.

Comparison of HLV with satellite RNAs and with the known viroids

The sequence of HLV was compared with the known sequences of viroids and circular satellite RNAs. It was found that HLV exhibits no significant sequence similarities with any of the circular satellite RNAs (virusoids). The sequence similarities of HLV with PSTV, HSV and CCCV (18) ASSV (19) and ASBV (20) which are the prototypes of the corresponding viroid groups is presented in Table 1. This overall calculation shows that HLV shares a similarity of 54% with CCCV whereas the similarity with all the other viroids ranges between 36%-51%. For a more detailed analysis of these similarities, the sequences of HLV, PSTV, HSV and CCCV were arranged in a co-linear way (Fig. 4). For this purpose the matching sequences of the upper and lower part of the central conserved region (CCR) of all these viroids were aligned first. Since all these viroids differ in their chain length, the maximal matching of at least five or more residues of their non-CCR sequence was achieved by introducing appropriate gaps.

This direct sequence comparison demonstrates that a series

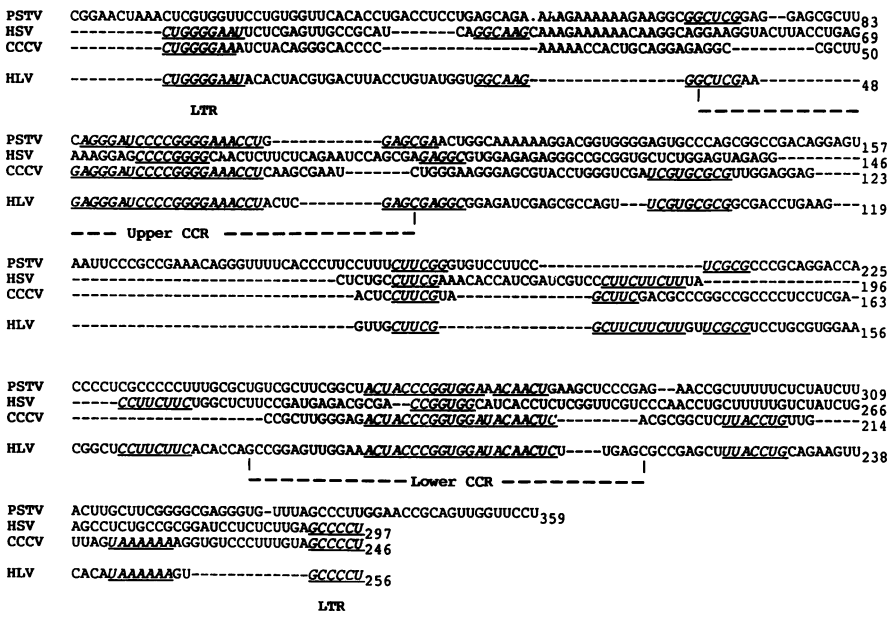


Figure 4. Sequence of HLV as compared with the sequence of PSTV, HSV and CCCV. (--): Gaps introduced to obtain maximal matching between the sequence of HLV and the sequence of the other prototype viroids. The underlined sequences characterize motifs in which an uninterrupted stretch of 5 or more HLV-specific nucleotides is also found in at least one of three other prototype viroids. LTR: left terminal region; CCR: central conserved region.

of sequence motifs of HLV is also present in one or more of the other three prototype viroids. Of particular interest are the longer motifs which comprise more than twelve residues. One of those is found in the left terminal region (LTR) of HLV, HSV and CCCV whereas the others are present in the upper and lower strand of the CCR of HLV, PSTV and CCCV (Fig. 4).

When HLV is arranged and compared in the same co-linear way with apple scar skin viroid (ASSV) (19) and the recently discovered grapevine yellow speckle viroid (GYSV) (21) (data not shown) the longest HLV-specific sequence motifs in these two viroids are seven and eleven nucleotides long, respectively. This finding is not surprising because the CCRs of ASSV and GYSV differ considerably from that of HLV and the other

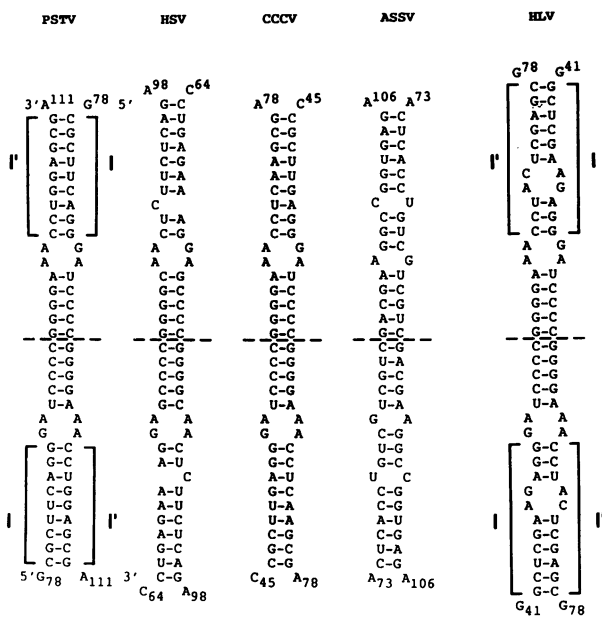


Figure 5. The putative processing configuration of PSTV (23) as applied to HSV, CCCV, ASSV and HLV as the prototypes of the other viroid groups. The point symmetric structures are divided by the indicated line into two identical parts each of which contains either one or two small interior loops. In PSTV the residues of the stem sequence forming the thermodynamically stable structure of the so-called hairpin I (36) are indicated by I and I'. In HLV the regions indicated by I and I' refer to the corresponding sequence motifs shown in Fig. 4.

viroids. HLV does not contain the oligo-A stretch in the left hand part of the rod-like secondary structure of all these other viroids (22). The low sequence similarity between HLV and avocado sun blotch viroid (ASBV) (20) of 36% is also found between ASBV and the other viroids (36-43%) and reflects the exceptional position of ASBV.

Other viroid-specific structural features of HLV

When the HLV sequence was analyzed for other viroid-specific structural features, it was found that its dimeric form can be arranged into a configuration, where the 28 predominantly C/G residues of the upper part of the two CCRs can form a highly base-paired structure (Fig. 5). This configuration is practically identical to that proposed to guarantee the proces-

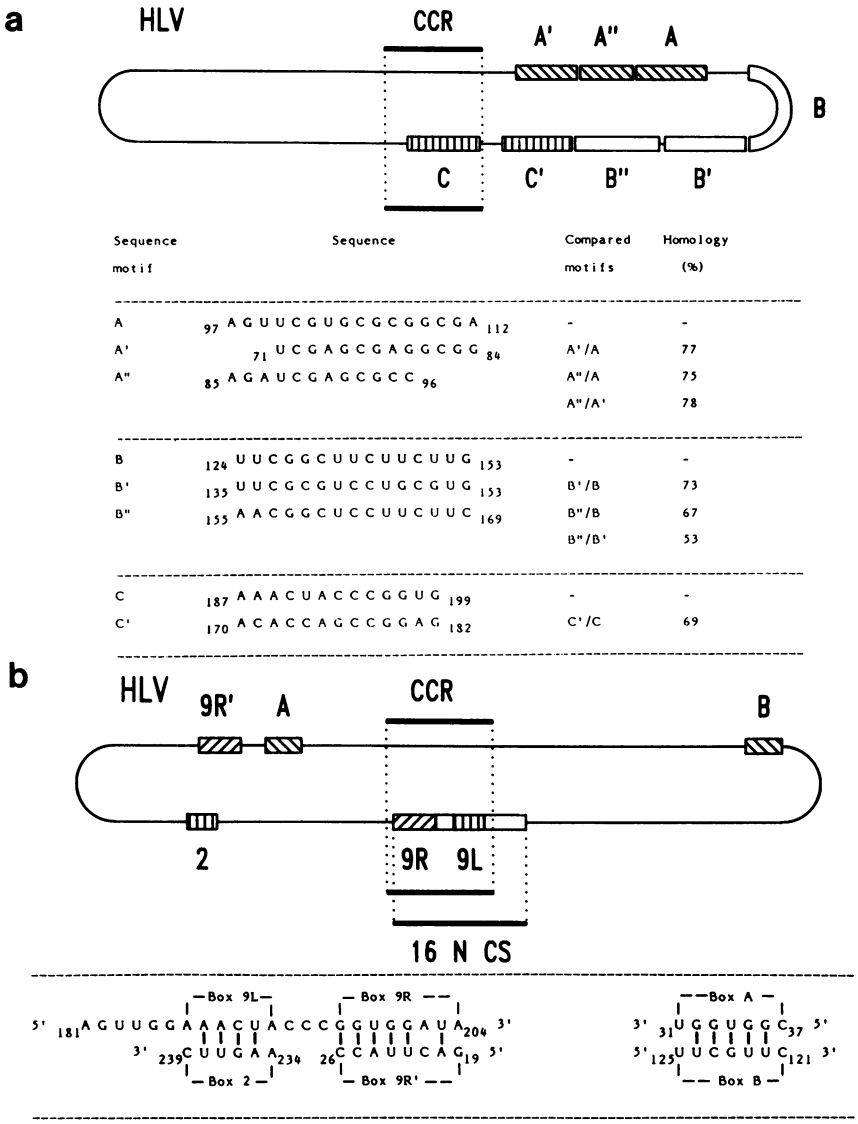


Figure 6. Viroid-specific structural features of HLV.
a: Schematic representation of the possible duplication of sequence motifs A, B and C in the HLV molecule, and their comparison by alignment of the corresponding nucleotides. The incomplete similarity between the motifs A, B and C with A', A'', B', B'' and C', respectively, could be due to mutational events that occurred during or after their duplication.
b: Schematic representation of the relative positions of the group I intron-like "boxes" in the HLV molecule and their possible base-pairing scheme.

sibility of oligomeric PSTV RNA and of the other viroid RNAs with an identical or structurally very similar CCR (23). Like all other true viroids, dimeric HLV RNA cannot be arranged into the so-called "hammerhead" structure which is characteristic for circular satellite RNAs (virusoids) and for the exceptional ASBV (24) and a structural precondition for their autocatalytic cleavage *in vitro* (25).

HLV also contains sequence motifs that could have arisen by sequence duplication (Fig. 6a) as proposed for CCCV (18) and PSTV (22) under the assumption that viroids may have evolved from smaller entities by such duplication events (22). Finally, the various group I intron-like "boxes" present in PSTV and the other viroids (26, 27) are also found in HLV (Fig. 6b). Due to the presence of these intron hallmarks in HLV, the postulated evolutionary or functional relationship between viroids and introns can also be extended to HLV. All these viroid-specific features strengthen the case for the viroid nature of HLV.

Infectivity of HLV

The main characteristic of viroids is their infectivity i. e. their ability to initiate replication and the production of viroid progeny when inoculated onto suitable host plants (28, 29). Therefore infectivity assays with 2M LiCl-soluble RNA from HLV infected hop cones of the cv "Record" were performed. Mock-inoculated and untreated hop plants were used as control, and all plants were maintained in the greenhouse for a period of six month. To monitor the replication of HLV, leaf samples were collected every twenty days and analyzed by dot spotting. Newly synthesized HLV was first detected six weeks post inoculation (p.i.) in three out of the inoculated eight hop plants. Four months p.i. six of these eight plants proved to be HLV-infected. When *in vitro* synthesized dimeric HLV(+)RNA was bioassayed, HLV was found to accumulate in two out of eight inoculated hop plants which was first detectable six weeks p.i. It is noteworthy that no disease symptoms whatsoever could be observed in any of the HLV-infected hops which is in accordance with our field observation.

In search for a herbaceous host plant more suitable for experimentation with HLV than hops, we have also assayed

Table 2 The distribution of HLV and HSV in individual hop cultivars (cvs) from the main hop growing areas of the world as determined by dot spot hybridization with HLV- and HSV- specific RNA probes

Origin of sample	Index of infection[a]	
	HLV	HSV
Europe		
Belgium	4/4	0/4
Czechoslovakia	3/3	0/3
England	15/17	0/17
France	2/2	0/2
East Germany	1/1	0/1
Hungary	4/4	0/4
Poland	1/1	0/1
Portugal	1/1	0/1
Russia	2/2	0/2
Spain	1/2	0/2
Yugoslavia	11/13	0/13
West Germany	14/14	0/14
Asia		
Japan	3/3	1/3
South Korea[b]	1/1	1/1
China	2/2	0/2
America		
USA (Oregon)	12/12	0/12
USA (Washington)	7/7	0/7
Africa		
South Africa	1/2	0/2
Oceania		
Australia	0/1	0/1
New Zealand	0/1	0/1
Total number of samples[c]	85/93	2/93
Total number of cultivars[c]	73/80	1/80

a: The numerator gives number of viroid-infected hop cvs, the denominator the total number of the individual cvs tested.
b: The tested cv "Kirin 2" was originally introduced to Korea from Japan
c: The total number of samples and of cvs differs because certain cultivars are grown in more than one country. In some cases a mixed commercial sample from an individual cv was used for analysis.

cucumber, tomato, *Chrysanthemum*, *Benincasa hispida*, *Gynura aurantiaca* and *Nicotiana glutinosa*. So far, we have not been able to observe the replication of HLV in any of these plants. This is partly documented in Fig. 2, where the signals in lane

2 and 9 clearly demonstrate that only HSV but not HLV is capable of infecting *Benincasa hispida* when the naturally occurring mixture of both viroids in Japanese hops is used as inoculum. Further studies on the natural and experimental host range of HLV are in progress.

Worldwide distribution of HLV

Since we have cloned HLV cDNA and HSV cDNA (9), we were able to synthesize *in vitro* the corresponding (-)RNAs and use those for the reliable detection of HLV and HSV. This allowed us to extend our study and to analyze by dot spot hybridization several hundred samples from the main hop growing areas around the world. These investigations (to be published elsewhere in detail) have, so far, shown that HLV is widespread in practically all hop cultivars around the globe, whereas HSV is, indeed, found only in Japanese hops which is also grown in Korea and may contain HLV in addition (Table 2). So far, we could not detect HLV or HSV in a hop sample from Australia (cvs "Pride of Ringwood") and from New Zealand (cvs "Sticklebrack"). However, a final conclusion on the absence of viroids in hops in both countries can only be drawn when more samples have been analyzed.

DISCUSSION

The viroid nature of HLV

We have accumulated structural and biological data which clearly demonstrate that the cirular RNA we have found to occur worldwide in apparently healthy hops is a viroid, tentatively named hop latent viroid (HLV). Its circular RNA in the viroid size range has the potential to asssume by intramolecular base-pairing the viroid-specific rod-like structure (Fig. 3). HLV not only contains the central conserved region characteristic for most of the presently known viroids but it also shares certain other sequence motifs with those (Fig. 4). Dimeric HLV RNA can potentially form the configuration that has been proposed to guarantee the processing of the oligomeric viroid RNA intermediates to the mature monomeric viroid circle (23). Finally, we have ascertained that the naturally occuring and the molecularly cloned HLV is infectious in that it initates

replication and accumulation when mechanically inoculated onto hops.So far no other natural or experimental host plants have been found in which HLV replicates.

HLV as the first member of a new viroid group

At present the sequence of about a dozen different viroids and of about twenty isolates and sequence variants thereof is known (28, 29). On the basis of obvious differences in their sequence they can be arranged into five groups represented by potato spindle tuber viroid (PSTV), coconut cadang cadang viroid (CCCV), hop stunt viroid (HSV), apple scar skin viroid (ASSV) and the exceptional avocado sunblotch viroid (ASBV), respectively, as prototypes of these groups. With the exception of ASBV, whose viroid nature may be questioned (23) the sequence similarity between the four group prototypes ranges between 39-60%, whereas it ranges between 65-100% when the members within a group are compared (e.g. PSTV with its group members CEV, CSV, tomato apical stunt viroid (TASV) and tomato planta macho viroid (TPMV) (30) or CCCV with coconut tinangaja viroid (CTiV) (31)). According to these criteria it is justified to regard HLV as first member of a new viroid group, because its sequence similarity with all other viroids is 36-54% (Table 1), and thus in the similarity range existing between the other group prototypes.

The latency and worldwide distribution of HLV

Our survey has shown that HLV occurs in almost all hop cultivars growing around the globe. In contrast to HSV it has remained undetected so long because it seems to be truely latent in hops. Only very recently in two symptomless commercial Spanish hop varieties a circular viroid-like RNA has been found and partially characterized (32) which resembles in all probability HLV.

Hops are propagated vegetatively, and there is an extensive exchange of hop varieties amongst growers and breeders in the various countries and continents. In the past, the hop plants to be used for vegetative propagation were mainly selected by visual inspection. Thus, any symptom-bearing plants carrying a pathogenic virus or viroid were prophylactically excluded from further propagation. Since the remaining symptom-free

stock plants could, so far, not be analyzed for the presence of latent viroids or cryptic viruses such plants have been unwittingly propagated and distributed worldwide like truely healthy plants. The localized spread of HLV and other cryptic agents in the field or greenhouse can proceed quite efficiently from infected to healthy plants via foliar and vascular wounding by cutting tools and pruning machinery contaminated with infectious plant sap (28, 29).

Latent viroids should not be underestimated as pathogens because they are a potential hazard for other crop plants to which they can be easily transmitted by various means and then cause severe damage. This is clearly demonstrated in the previously reported cases of columnea latent viroid (29, 33) and of HSV as one of the latent grapevine viroids (34, 35). Both viroids cause severe disease symptoms when transmitted by various means from their non-reactive hosts to tomato and potato and to hops, respectively. Moreover, latent viroids may posses an indirect pathogenic potential in that they might initiate or aggravate disease when additional infections with microbial, viral and other viroid agents take place or when adverse conditions arise during culture. In future these threats can be countered to some extent by routinely applying the sensitive, specific and rapid diagnostic tests as utilized in this report. In this way all mother plants and root stock to be used for vegetative propagation can be indexed for the presence of latent viroids and the viroid-free plants can then be used for further propagation. Our study has shown that such viroid-free plants can be found in populations of infected hop cultivars.

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