Ultrafast sequencing of oligodeoxyribonucleotides by FAB-mass spectrometry

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ABSTRACT

   A fully instrumental method is described for the bidirectional
sequencing of oligodeoxyribonucleotides. The method makes use of
the negative ion fragmentation patterns of fast atom bombardment
mass spectrometry. It is less time consuming than any other se-
quencing procedure known to date. Since one sequencing run takes
as little as one hour, this new method is anticipated to cut
down considerably the time required for the controlled synthesis
of oligodeoxyribonucleotides of (currently) up to ten nucleotide
units in length.

INTRODUCTION

   Synthetic oligonucleotides have proved extremely useful in
modern biosciences. In particular the chemical synthesis of pre-
defined nucleotide sequences has supported and accelerated the
recent development of gene technology. This has stimulated the
development of new and quicker synthetic techniques such as bet-
ter coupling procedures[1,2], highly selective and mild agents for
deprotection[3,4], and the use of suitable solid supports[5-7]. These
syntheses can be performed in a matter of days and as a conse-
quence the longer part of the work is now the careful analysis of
the deprotected oligonucleotides. Sequencing is usually done by
'chemical degradation'[8] or 'wandering spot'[9] procedures after ra-
dioactive labelling. Here we describe a completely instrumental
method for a much faster (less than one hour) and unambiguous se-
quence analysis of oligodeoxyribonucleotides up to at least ten
units in length using the negative ion fragmentation patterns of
fast atom bombardment mass spectrometry (FAB-MS)[10].

MATERIALS AND METHODS

   Oligodeoxyribonucleotides were synthesized following, in gene-

ral, established phosphate triester procedures. Benzoyl, anisoyl
and isobutyryl groups were used for the protection of the hetero-
cyclic bases, 4-methoxy-trityl for the protection of the 5'-OH,
and 2-chlorophenyl and 2,2,2-tribromoethyl for the protection of
the phosphate functions. Condensation reactions were performed in
solution with 2,4,6-tri-isopropylbenzene-sulfonyl-3-nitro-1,2,4-
triazolide as condensing agent. For the final work-up the unpro-
tected oligomers were formed by treatment with 10 % pyridine in
concentrated ammonia followed by 80 % acetic acid. Homogeneity
higher than 99 % was routinely achieved after ion exchange chro-
matography on Sephadex A-25 in the presence of 7 M urea and sub-
sequent desalting. This was carefully checked by reverse phase
HPLC, polyacrylamide-gel electrophoresis in the presence of chain
length markers[11] and two-dimensional finger printing.

The mass spectra were recorded on a Kratos MS 50 S with a high
field magnet (mass range ca. 3000 at 8 kV) and a Kratos FAB sour-
ce. The atom gun used xenon and produced a beam of neutral atoms
at 8-9 keV. A solution of the triethylammonium salt of each oli-
godeoxyribcnucleotide (4-5 µl, containing 1-1.5 OD$_{260}$, ca. 10 nmol)
was injected into the glycerol matrix (ca. 2 µl) present on the
FAB copper probe tip. The water was removed in the direct inser-
tion lock and the spectra were recorded at a magnet scan rate cf
300 s/decade.

RESULTS AND DISCUSSION

The Figures 1a, 2a and 3a shcw the negative ion FAB mass spec-
tra of the oligodeoxyribonucleotides d(A-C-T-C-G-A-T-G), d(G-C-G-
A-T-C-G-C), and d(G-A-A-G-A-T-C-T-T-C). The highest mass is the
(M-H)$^-$-ion. The corresponding doubly charged ion is found at half
mass. The mass difference between twc markers is indicative of a
particular nucleotide. Those above the spectra mark fragment ions
with 5'-phosphate ends (5'-P sequence ions) and those belcw mark
fragment ions with 3'-phosphate ends (3'-P sequence ions). This
is also represented by the shorthand structures in the Figures
1b, 2b and 3b. The dotted lines show the main fragmentations with
attached mass, those above corresponding to the 5'-P sequence
ions and those below to the 3'-P sequence ions.

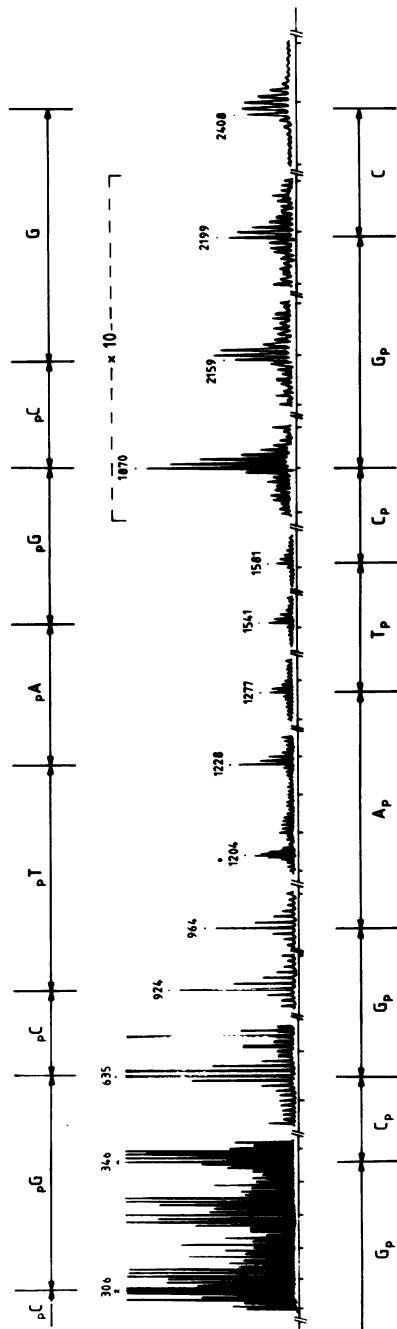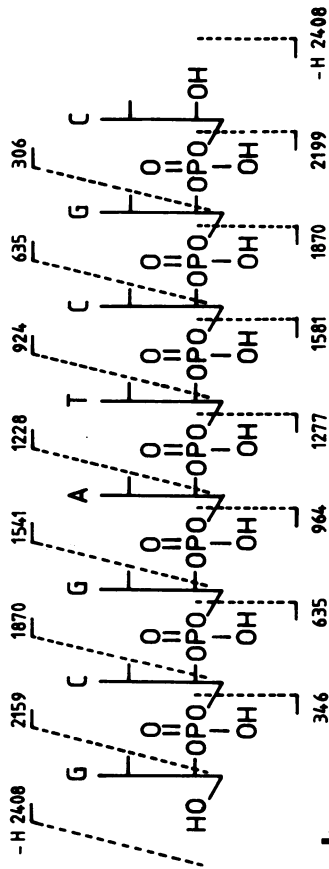A similar but more complex fragmentation behaviour was recent-
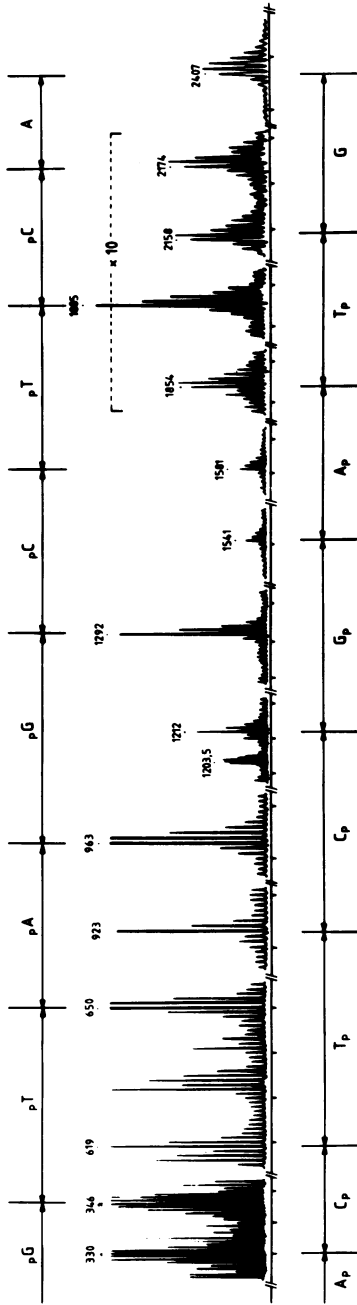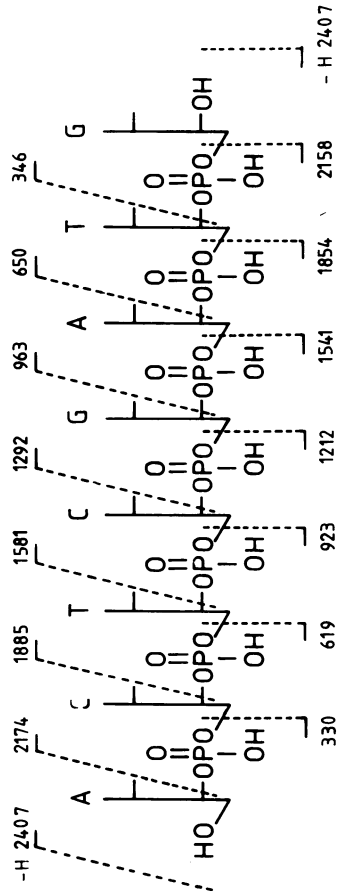
Figure 1a: Relevant regions of the negative ion FAB mass spectrum of d(A-C-T-C-G-A-T-G) excluding the glycerol matrix.

Figure 1b: The corresponding shorthand structure with those bonds marked that on breakage give rise to the main fragment ions.

Figure 2a: Relevant regions of the negative ion FAB mass spectrum of d(G-C-G-A-T-C-G-C) excluding the glycerol matrix.

Figure 2b: The corresponding shorthand structure with those bonds marked that on breakage give rise to the main fragment ions.
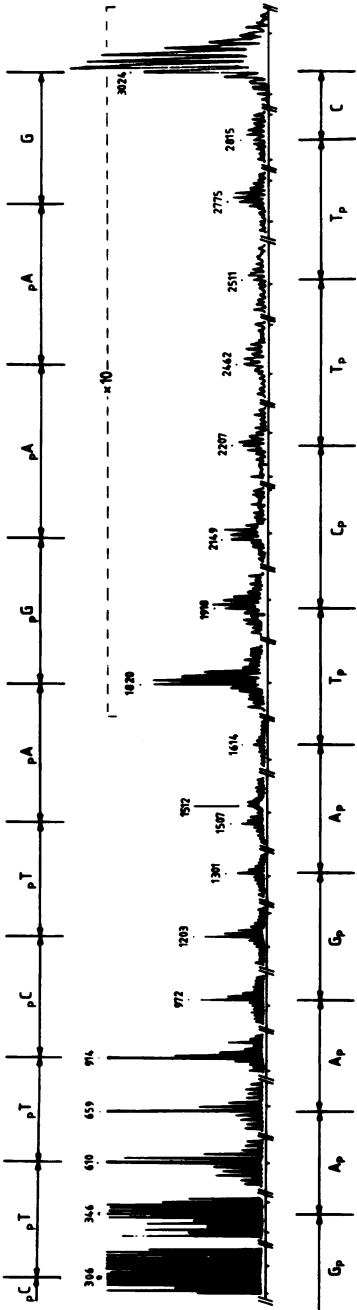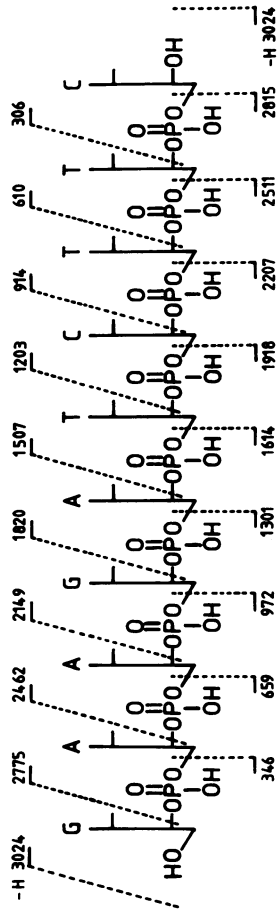
Figure 3a: Relevant regions of the negative ion FAB mass spectrum of d(G-A-A-G-A-T-C-T-T-C) excluding the glycerol matrix.

Figure 3b: The corresponding shorthand structure with those bonds marked that on breakage give rise to the main fragment ions.

ly described for negative ion ^{252}Cf-plasma desorption mass spec-
trometry of fully protected oligonucleotides[12]. These authors al-
so state, that oligonucleotides containing the naturally occuring
phosphodiester internucleotidic links exhibit a still more com-
plex fragmentation pathway, and that molecular ion yields are se-
verely attenuated. On the contrary in our FAB mass spectra we
only observe sequence ions accompanied by some minor -18 peaks.
This exclusive formation of specific 5'-P and 3'-P sequence ions
gives rapid sequence information simply by counting mass diffe-
rences between consecutive sequence ions of the same type. The
discrimination of these two types of sequence ions is possible,
because those ions with the same number of nucleotide units show
a consistent difference in peak intensities. Since the bond in-
volving the 3'-O atom, connected to a secondary carbon of the su-
gar moiety, is more labile than the one involving the 5'-O atom,
connected to the primary carbon of the sugar, the 5'-P sequence
ions are without exception more intense than the corresponding
3'-P sequence ions.

In practice the determination of the nucleotide sequence pro-
ceeds as follows: Starting from the (M-H)$^-$ peak all sequence ions
are classified as either 5'-P ions or 3'-P ions according to
their intensities. Then the exact mass differences between neigh-
bouring peaks of the same family are determined by counting to
give their corresponding nucleotides. The complete base sequence
of the oligonucleotide can then be read independently twice,
starting either from the 5'- or from the 3'-end.

It is obvious, however, that all fragments with the same base
composition have the same mass for both the 5'-P and the 3'-P se-
quence ions. Thus, the spectrum of d(G-C-G-A-T-C-G-C) shows only
one peak for both terminal dinucleotide ions. However, despite
any ambiguity in this mass area, the evaluation of the actual se-
quence does not create any problem. It is clear from the spectrum
as a whole that only one peak shows up in a wide mass range. Mas-
ses and peak intensities of the higher oligonucleotide ions clear-
ly confirm that, reading the sequence from the 5'-end, position 2
is C and position 7 is G and the single peak at mass 635 repre-
sents both dinucleotide ions.

The FAB-MS negative ion fragmentation pattern can well be com-

pared to the result of the simultaneous action of two exonucle-
ases partially digesting oligonucleotides from either end. How-
ever, it is unlikely that there will ever be any enzymatic pro-
cedure of comparable speed and resolving power.

We have performed mass spectrometric sequence analysis of
various oligonucleotides. The method is of general applicability
and is now routinely used in our laboratory. Thus, negative ion
FAB-MS is a quick and reliable method for the bidirectional se-
quence analysis of unprotected oligodeoxyribonucleotides up to
at least ten base units in length.

Future instrumental technology should enable mass ranges up to
and above 5000^{13} to be exploited with the corresponding possibi-
lity of sequencing oligonucleotides of more than 16 base units.
Current improvements to the mass spectrometer will allow us to
sequence our longer oligonucleotides by this method. We antici-
pate that this method will help to speed up considerably the re-
liable synthesis of gene and other DNA fragments.

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