SEQUENATOR DETERMINATION OF THE AMINO ACID SEQUENCE OF APOVITELLENIN I FROM TURKEY'S EGG YOLK

Use of a stationary reaction cup during peptide bond cleavage

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1. Introduction

Apovitellenin I is a protein of low molecular weight ($\sim 10^4$) that is readily isolated from the major (i.e., low-density) lipoprotein of the yolk of avian eggs. The amino acid sequences of this protein from three species (duck [1], hen [2] and emu [3]) show a high proportion of changes, about 20% of the total residues, which include rare substitutions. We report here the sequence of a fourth protein, turkey's apovitellenin I. This sequence was determined because the turkey is biologically more closely related to the hen than to the duck or the emu. We have found, unexpectedly, that the turkey's apovitellenin I is no closer to that of the hen in sequence and in other properties than it is to apovitellenin I of the other species.

During this work, for which a spinning-cup sequenator was used, we introduced a simplifying modification the cup was kept stationary during the acid cleavage of the peptide bonds. The advantages of this approach reside in elimination of the major source of movement of the film in the cup, and of the necessity for very accurate metering of the pentafluoropropionic acid to the cup. With this modification and using a single cleavage (except for the proline at residue 31) the first 69 residues of the intact protein were determined.

2 Materials and methods

Eggs from turkeys (*Meleagris gallopavo*) were from a commercial hatchery in New South Wales. The major yolk high-lipid, low-density lipoprotein was isolated by a procedure similar to that used for hen's eggs [4] with the difference that less concentrated salt was used (2 M NaCl instead of 4 M). The isolation and properties of this lipoprotein have been reported in [5].

The lipid-free apoproteins were isolated from the lipoprotein with chloroform–methanol by the two methods described in [4]. Apovitellenin I was separated by gel-filtration chromatography in acidic urea as shown in fig.1. This figure also shows a phenomenon not observed with the other avian species, namely, when the apoprotein mixture was allowed to stand in urea the proportion of apovitellenin I that could be separated by chromatography decreased and there were signs of aggregation that was not disrupted in urea. There was also a decrease in the amount of apovitellenin I if the eggs had been stored for longer than a few days at 2°C. This behaviour made it difficult to assess the proportion of turkey's apovitellenin I in the apoprotein mixture; the maximum amount isolated was about 55% of the total.

According to sedimentation equilibrium measurements in 6 M guanidine–HCl plus mercaptoethanol, turkey's apovitellenin I has mol. wt 9500, and according to gel electrophoresis in sodium dodecyl sulphate is $\sim 10^4$ mol wt. Amino acid analysis after 24 h and 72 h hydrolysis at 115°C in methanesulfonic acid [6] indicated that the molecule contained the following residues, the figures in parenthesis were obtained

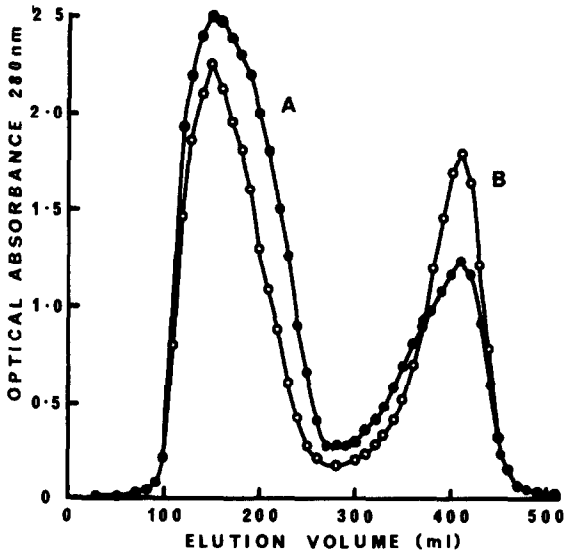

Fig.1. Chromatography on a column of Sephadex G-100 fine (35 × 3.5 cm) of two samples (~30 ml, 1%, w/v) of total apoprotein from turkey's lipoprotein. The solvent was 6 M urea, 0.25 M HCl (pH 3.3) 20°C. One sample (open circles) was applied within 12 h of preparation. The other (filled-in circles) was left for 3 days at 2°C. Peak A contained a mixture of proteins of high molecular weight. Peak B contained apovitellenin I which was further purified on a column of Sephadex G-75

after hydriodic acid hydrolysis for 22 h at 140°C [7]· Asx 7.0, Thr 5.5 (5.7), Ser 3.9, Glx 8.7 (9.0), Pro 2.2, Gly 3.4, Ala 6.8, Val 6.1 (7.1), Met 2.0, Ile 6.0 (6.8), Leu 7.0 (6.9), Tyr 3.6 (3 9), Phe 3.0, Lys 5.0, Trp 1.9, Arg 7.1. Amide analysis using amino acid analyser [8] gave 7 residues (6.8 found).

The C-terminal cyanogen bromide fragment was prepared as described previously for duck's apovitellenin I [1]. After hydrolysis in methanesulfonic acid amino acid analysis gave· Asx 1.0, Thr 0.9, Glx 2.0, Gly 1.0, Val 0.9, Ile 0.9, Leu 1.0, Tyr 1.8, Trp 0.9, Lys 2.9.

The sequenator analysis of this peptide (200 nmol) was performed as in [1] with cytochrome *c* (1 mg) and dithiothreitol (1.5 mg) as carriers.

The N-terminal sequence determination of the protein (0.4 μmol) was carried as far as residue 69 by using the sequenator as in [1] with the following differences

(a) Ethyl acetate extraction was discontinued at degradation cycle 50 and replaced by extraction with 1,2-dichloroethane–benzene mixture (3.1) to ensure retention of peptide in the cup;

(b) For acid cleavage with pentafluoropropionic acid the cup was stationary for the following stages. acid addition to the bottom of the cup (14 s), rough vacuum stage (4 s), reaction (240 s), rough vacuum stage (5 s). The first rough vacuum stage serves to remove acid from the inlet line and evaporate it from the base of the cup; the film completely wets out when the vacuum valve is shut and the nitrogen line is opened. The second short rough vacuum step precedes a longer vacuum drying period with the cup spinning and serves to dry the film partially before the cup drive is actuated.

A single cleavage was given throughout except for residue 31 (proline) when a second cleavage cycle was introduced.

Residues 40 through 70 were confirmed by sequenator analysis of a fragment (comprising residues 40 through 82) obtained after 0.0125 M hydrochloric acid cleavage at 108°C for 2 h [9] and Sephadex G-50 (fine) chromatography in 75% formic acid.

3. Results and discussion

With the low pressure nitrogen system used to transfer reagent from the reagent bottle to the reaction cup in the protein sequenator, variations in delivery volume can occur with changes in volume of the reagent. The amount of acid added is particularly critical because it can affect the cleavage yield as well as shift the protein film as it rises up the cup wall. The modification we describe above, in which the cup is stationary for part of the degradation cycle, removes these two problems because the acid is volatilised from the bottom of the cup onto the protein film on the wall. This reduces the stringency of the procedure; it also minimizes the amounts of volatile reagents that need to be added – usually they tend to be partially evaporated from the rotating surface. Certainly the efficiency of the cleavage reaction was not impaired and repetitive yields for a single cleavage reaction (based on HPLC of leucine derivatives at residues 2, 9 and 11 of apomyoglobin) were 97%

Besides methanesulfonic acid hydrolyses [6],

hydriodic acid hydrolysis of the protein [7] was employed in this work to ensure satisfactory amino acid analyses for valine and isoleucine. These data indicated that there was 1 more serine and 1 less valine residue than was found in the sequence. Hence, the partial sequence analysis of the HCl cleavage fragment (residues 40–82) was made and this established that residue 47 (Val→Ser) initially had been assigned incorrectly due to valine appreciably overlapping from residues 45 and 46 into step 47 and the non-detection of the serine residue at step 47.

Table 1 gives the sequence of turkey's apovitellenin I determined using this procedure. The codon differences from the previously reported sequences of hen's and duck's proteins are indicated. The variations are in line with those found in a previous comparison with emu's apovitellenin I [1]. The turkey's sequence also has an unusual amino acid replacement – a serine at residue 47 instead of either valine or isoleucine. The sequence of turkey's apovitellenin I is not especially close to that of the hen. In fact it would be difficult to tell from table 1 that the turkey and the hen are from the same order (Galliformes) that diverged from the Anseriformes (including ducks) about 60 million years earlier than the hen–turkey divergence [10]. The reasons for this dissimilarity in sequence are not clear at present. Possibly, as suggested in [4], hen's apovitellenin I is unusual. There may, for example, be more than one protein. A recent report of the sequence of a low molecular weight protein (apoVLDL-II) from a lipoprotein of laying-hen's blood [11] gives several differences from that of hen's apovitellenin I [2] although the proteins are definitely related. This sequence of the plasma protein is, however, even further from that of the turkey's egg yolk. It is

Table 1
Amino acid sequence of apovitellenin I from turkey egg yolk and homology with apovitellenin of duck and hen

	1				5					10					15				20
Turkey	Lys	Ser	Ile	Phe	Glu	Arg	Asp	Arg	Arg	Asp	Trp	Leu	Val	Ile	Pro	Asp	Ala	Val	Ala	Ala
Duck	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Hen	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0

	21				25					30					35				40
Turkey	Tyr	Ile	Tyr	Glu	Ala	Val	Asn	Lys	Met	Ser	Pro	Arg	Ala	Gly	Gln	Phe	Leu	Val	Asp	Ile
Duck	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2
Hen	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1

	41				45					50					55				60
Turkey	Ser	Gln	Thr	Thr	Val	Val	Ser	Gly	Thr	Arg	Asn	Phe	Leu	Ile	Arg	Glu	Thr	Ala	Arg	Leu
Duck	1	0	0	1	0	0	2	0	0	0	1	0	0	0	0	0	0	1	2	0
Hen	0	0	0	0	0	0	2	0	1	0	0	0	0	0	2	0	0	0	0	0

	61				65					70					75					80
Turkey	Thr	Ile	Leu	Ala	Glu	Gln	Leu	Met	Glu	Lys	Ile	Lys	Asn	Leu	Trp	Tyr	Thr	Lys	Val	Gln
Duck	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hen	0	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1

	81	82
Turkey	Gly	Tyr
Duck	0	0
Hen	0	0

Numerals indicate the minimum number of base changes necessary to convert any amino acid into its corresponding residue in the duck [1] or hen [2] sequences. Underlined residues show positions of identity in all apovitellenins. Residues 1–69 were determined on the intact protein, residues 69–82 on the C-terminal cyanogen bromide fragment and residues 40–72 were confirmed by analysis of a dilute acid cleavage product

possible that work now in progress on apovitellenin I from more closely-related species will help explain our present results.

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