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# MOLECULAR WEIGHT AND BEHAVIOR OF LIPOVITELLIN IN UREA SOLUTIONS*

G. BERNARDI** AND W. H. COOK

*Division of Applied Biology, National Research Council, Ottawa, Ont. (Canada)*

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SUMMARY

Lipovitellin in 4 *M* urea has a molecular weight of $2.0 \pm 0.2 \cdot 10^5$, which is half that observed in 1 *M* NaCl. The two sub-units present in urea are undistinguishable on the basis of their ultracentrifugal properties. β-Lipovitellin dissociates at lower urea concentrations than α-lipovitellin. Although lipovitellin is denatured by urea and suffers a loss of lipid in this solvent, its dissociation is partly reversible on reducing the concentration of urea.

## INTRODUCTION

Recent studies[1,2] have shown that lipovitellin and its two electrophoretic components (α- and β-lipovitellin) all sediment as a single component in 1 *M* NaCl, and have a molecular weight of $4.0 \cdot 10^5$ in this solvent. In alkaline solvents, however, lipovitellin partially dissociates to yield a second slower sedimenting component[1]. A monodisperse solution of these sub-units is required to estimate their molecular weight but, since

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** Present address: Centre de Recherches sur les Macromolécules, Strasbourg, France.

complete dissociation was not obtained at pH values as high as 10.9 (see ref. 2), alkaline solvents were considered unsatisfactory. Since lipovitellin has been shown[3] to dissociate completely into a slower sedimenting component in 4 *M* urea solutions, this solvent was used in the present study to estimate the molecular weight of the sub-units.

## METHODS AND MATERIAL

The physical and chemical methods employed have been described in earlier papers[1,2]. In determining total lipids[2] this fraction was re-extracted with chloroform, as urea is less soluble in this solvent than in ethyl ether. Diffusion measurements in urea solutions were limited to those with schlieren optics in a Spinco Model H apparatus or in a synthetic boundary cell in the ultracentrifuge[2], as the high refractive index of 4 *M* urea solutions precluded the use of interferometric methods. Several attempts to adjust the optical paths lead to distorted fringes. All physical measurements were made at 20°.

Lipovitellin was prepared by the magnesium sulphate method[1] and $\alpha$- and $\beta$-lipovitellin by chromatographing the high density fraction of egg yolk on hydroxy-apatite columns[2]. Most of the measurements were made on lipovitellin prepared by precipitation, since $\alpha$- and $\beta$-lipovitellin have the same molecular weight as the unfractionated material, and the sub-units cannot as yet be separated. Purity of these preparations was confirmed by sedimentation and electrophoretic analyses.

Lipovitellin and its $\alpha$- and $\beta$-components were adjusted to the urea concentrations required by dialysing the starting solution (in 1 *M* NaCl or phosphate buffer, respectively) against urea solutions containing either 0.25 *M* NaCl or veronal buffer (pH 9.0 and 0.3 $\mu$).

## RESULTS

The conversion of lipovitellin to the slower component was essentially complete in 24 h in solvents containing 4 *M* urea (Fig. 1B). At lower urea concentrations two components were evident (Fig. 1A), and the quantity of the slower sedimenting component increased slightly with time over several days. Exposure to solvents containing 4 *M* urea for periods of one month caused only a slight decrease in the sedimentation coefficients of the slow component. No differences were detected in the behaviour of samples in urea–veronal (pH 9.0) and in urea–0.25 *M* NaCl.

When 4 *M* urea was diluted by dialysis to 2 *M* urea, a fast sedimenting component was again evident (Fig. 1C). This indicates partial reversibility, although the proportion of fast component is somewhat less than in material exposed only to 2 *M* urea (Fig. 1A). On further reduction of the urea concentration to 1.5 *M*, a partial precipitation of lipovitellin occurred. Essentially complete precipitation occurred when the urea was entirely removed by dialysis against veronal buffer (pH 9.0), originally a good solvent for lipovitellin. This loss of solubility and the formation of gels when the protein concentration exceeded about 3% show that lipovitellin is denatured by 4 *M* urea.

Only a few experiments were made with separated $\alpha$- and $\beta$-lipovitellin. $\beta$-Lipovitellin is largely dissociated in 1 *M* urea (Fig. 1D) and the fast component had almost disappeared in 2 *M* urea (Fig. 1E). The fast component, however, still predominates

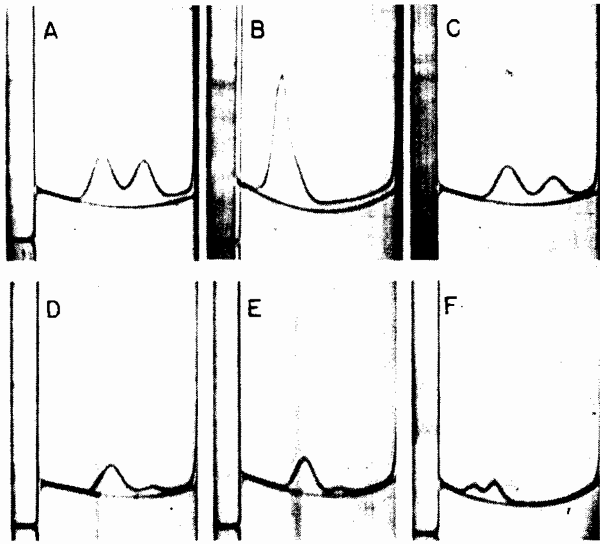


Fig. 1. Sedimentation patterns, in double sector cell at 50,740 rev./min, selected to show resolution in the different solvents: A, lipovitellin in 2 *M* urea + 0.25 *M* NaCl; B, lipovitellin in 4 *M* urea + 0.25 *M* NaCl; C, lipovitellin in 4 *M* urea + 0.25 *M* NaCl as dialysed back to 2 *M* urea + 0.25 *M* NaCl; D, β-lipovitellin in 1 *M* urea + 0.25 *M* NaCl; E, β-lipovitellin in 2 *M* urea + 0.25 *M* NaCl; F, α-lipovitellin in 2 *M* urea + 0.25 *M* NaCl.

in α-lipovitellin at this urea concentration (Fig. 1F). The relative degree of dissociation of the two lipovitellin fractions at different urea concentrations therefore parallels their behaviour with increasing pH values[2].

Electrophoretic analyses in 4 *M* urea were inconclusive because it was difficult to maintain stable boundaries. In a few experiments two boundaries were evident.

The results of the chemical analyses are summarized in Table I for lipovitellin that had not been treated with urea[2], and for material in 4 *M* urea. The lipovitellin dissolved in urea solutions contained only 12% total lipid, as compared with 19%

TABLE I

SUMMARY OF CHEMICAL ANALYSES ON LIPOVITELLIN*

| *Constituent* | | *1 M NaCl*** *pH approx. 7.0* | *4 M urea in veronal buffer, pH 9.0* | *4 M urea in 0.25 M NaCl, pH approx. 7.0* |
|---|---|---|---|---|
| Total lipids | % | 19 | 12 | 12 |
| Phospholipids | | | | |
| (lipid P × 25) | % | 12.5 | 6.75 | 9.00 |
| (chromatography) | % | 11.6 | 7.1 | 8.3 |
| Non-phospholipids | | | | |
| (chromatography) | % | 7.4 | 4.9 | 3.7 |
| Total phosphorus | % | 1.05 | 0.85 | |
| Protein phosphorus | % | 0.50 | 0.52 | |
| As % of vitellin | | 0.62 | 0.59 | |
| Lipid phosphorus | % | 0.50 | 0.27 | 0.36 |
| As % of lipid | | 2.63 | 2.25 | 3.00 |

* Reported values are expressed as percent of lipoprotein, except as otherwise indicated.
** From BERNARDI AND COOK[2].

in samples dissolved in 1 $M$ NaCl. Since the lipid determinations were made on the dialysed solutions, the only evident explanation is that part of the lipid was lost during the initial dialysis in urea solvents. The somewhat smaller proportion of phospholipid found in samples treated with 4 $M$ urea at pH 9.0 may indicate hydrolysis but this cannot be considered conclusive. Since lipid losses of about 7% are comparable with the experimental errors of estimating molecular weight, further studies on the effect of urea on lipid content were considered beyond the scope of the present work. While urea solutions alter the protein molecule, the protein phosphorus content was not affected.

TABLE II

SUMMARY OF PHYSICAL MEASUREMENTS ON LIPOVITELLIN

| *Property* | *Solvent* | | |
|---|---|---|---|
| | *1 M NaCl* pH approx. 7.0* | *4 M urea in veronal buffer pH 9.0* | *4 M urea in 0.25 M NaCl pH approx. 7.0* |
| d$n$/d$c$ (concentration in g/ml) at 5780 Å, 25° | 0.181 | (0.198)** | 0.200 |
| $\bar{v}$ (ml/g) | 0.778 | (0.737)** | 0.744 |
| $s^c_{20,w}$ (Svedbergs) | 10.5 | 6.2 | 5.8 |
| d$s$/d$c$ (concentration in g%) | —0.35 | —0.19 | —0.33 |
| $D_{20,w}$ ($10^{-7}$ cm²/sec) | | | |
| Schlieren | | | |
| In electrophoresis cell | 2.9 (0.33%) | 2.8 (0.33%) | — |
| In ultracentrifuge | | 3.2 (→ 0) | — |
| d$D$/d$c$ (concentration in g%) | | 0.5 | — |
| Molecular weight (× $10^5$) | | | |
| From $s$ and $D$ | 4.0 | 2.0 | — |
| | | 1.8 | — |
| From ARCHIBALD's method*** | 4.0 | 2.1 (0.70%) | 2.2 (0.73%) |

* From BERNARDI AND COOK[2].
** From JOUBERT AND COOK[3].
*** Not corrected for concentration dependence.

The physical measurements are summarized in Table II, including the comparable figures for lipovitellin that had not been exposed to urea. The higher specific refractive increment (d$n$/d$c$) and lower partial specific volume ($\bar{v}$) of lipovitellin in urea, as compared with values obtained in sodium chloride solutions, confirm previous observations[3]. The diffusion coefficients are only slightly higher than that of lipovitellin in 1 $M$ NaCl. Molecular weights of 1.8 to $2 \cdot 10^5$ were computed from these coefficients. Slightly higher values obtained by ARCHIBALD's transient state method, not corrected for concentration dependence, were confirmatory.

## DISCUSSION

Lipovitellin dissociates in urea solutions in a manner that parallels its dissociation in alkaline solvents[1,2]. Although urea solutions denature lipovitellin, the dissociation in this solvent is also partly reversible, as the faster sedimenting component reappears on diluting 4 $M$ to 2 $M$ urea. Again, $\beta$-lipovitellin, which dissociates more completely than $\alpha$-lipovitellin at low pH values, shows the greatest degree of dissociation at low urea concentrations. Evidently most of the slow component of unfractionated lipovitellin in 2 $M$ urea is $\beta$-lipovitellin.

While the sub-units obtained in alkaline and urea solvents may have the same origin, the sedimenting entities are not identical. A loss of lipid occurs in urea solutions and this may result from the formation of urea adducts of the fatty acids. Actually, the reported chemical analyses are compatible with the loss of one fatty acid molecule from each molecule of triglyceride or phospholipid. The sedimentation coefficient ($s^\circ_{20,w}$) of the sub-units is 7.4 S in alkaline solution[2] and only approx. 6.0 S in urea, a difference that can be explained by the changes in the protein moiety caused by urea denaturation.

The molecular weights obtained in urea range from 1.8 to $2.2 \cdot 10^5$, which is half that of lipovitellin ($4.0 \cdot 10^5$) in *M* NaCl. Although the quantitative measurements cannot be made with the same accuracy in 4 *M* urea as in dilute buffer solution, lipovitellin evidently dissociates into two sub-units that sediment as a single boundary, but may not be identical in size or composition.

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