Evidence for Protein–Polysaccharide Complex Formation as a Result of Dry-heating of Mixtures

By K. Jumel, S. E. Harding, J. R. Mitchell, and Eric Dickinson[1]

DEPARTMENT OF APPLIED BIOCHEMISTRY AND FOOD SCIENCE, UNIVERSITY OF NOTTINGHAM, SUTTON BONINGTON, LOUGHBOROUGH, LEICESTERSHIRE LE12 5RD, UK
[1]PROCTER DEPARTMENT OF FOOD SCIENCE, UNIVERSITY OF LEEDS, LEEDS LS2 9JT, UK

1 Introduction

The complexes produced by the dry-heating of the globular protein bovine serum albumin (BSA) and the non-ionic polysaccharide dextran have been shown recently to have excellent emulsion stabilizing properties.[1,2] This short paper presents experimental data on the molecular weight of complexes formed by dry-heating mixtures of BSA and dextran T40 (4×10^4 daltons) as determined by gel permeation chromatography with light-scattering detection (GPC/MALLS) and ultracentrifugation.

2 Experimental

Protein–polysaccharide complexes were prepared by dry heating various molar ratios of BSA and dextran T40 at 60 °C for 3 weeks as described previously.[1] The samples were dissolved in phosphate/chloride buffer (pH 7.0, ionic strength 0.1 M). Weight-average molecular weights of samples of various molar ratios were determined by GPC/MALLS (sample concentration 3 mg ml^{-1}).[3] The BSA–dextran complex of molar ratio 2:1 in a solution of concentration 0.5 mg ml^{-1} was investigated under conditions of low-speed sedimentation equilibrium in a Model E analytical ultracentrifuge. The sedimentation coefficient of this same complex (sample concentration 2 mg ml^{-1}) was determined using a Beckman XL-A analytical ultracentrifuge.

3 Results and Discussion

Figure 1 shows the weight-average molecular weight M_w of various BSA–dextran complexes as determined by GPC/MALLS. We see that, of the various samples investigated, only the complex with a composition of 33 mol % dextran has a molecular weight substantially greater than that for the native protein. Table 1 compares the value of M_w obtained from GPC/MALLS for this 2:1 molar ratio complex with that from low-speed sedimentation equilibrium. Also shown for comparison are M_w values for the heat-treated pure BSA (same heating conditions). The results indicate that, although some aggregation of the BSA itself occurs during the

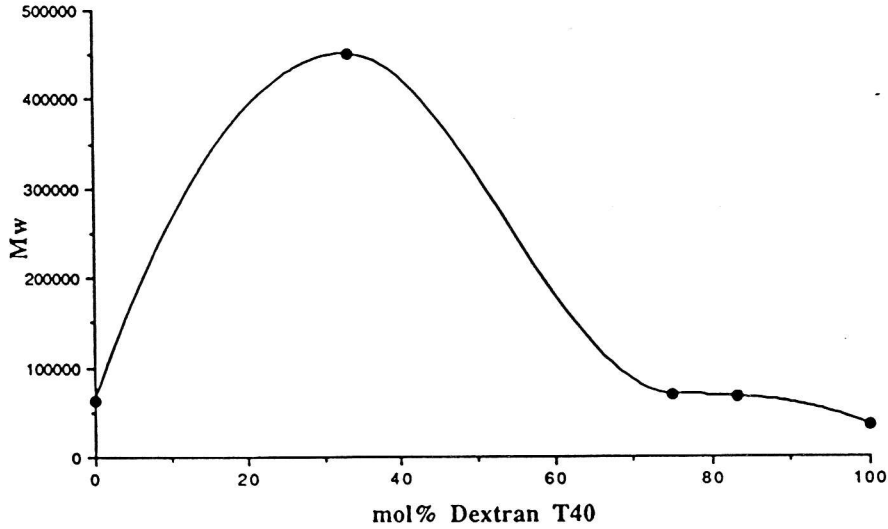

Figure 1 *Weight-average molecular weight M_w of complexes obtained by dry-heating of mixtures of BSA and dextran T40 at various molar ratios*

Table 1 *Molecular weight, sedimentation coefficients, and frictional coefficients*

Sample	M_w (daltons) *GPC/MALLS*	M_w (daltons) *Model E*	s_{20} (S)	$s_{20,w}$(S)	f/f_0
BSA native	62 700 ± 5 000	66 700[a]	3.93 ± 0.1	4.04 ± 0.1	1.3 ± 0.1
BSA heat-treated (3 weeks)	158 000 ± 10 000	130 000 ± 10 000	5.43 ± 0.1	5.59 ± 0.1	1.3 ± 0.1
BSA/T40 complex 2:1 ratio	450 000 ± 20 000	330 000 ± 20 000	4.85 ± 0.1	4.98 ± 0.1	3.4 ± 0.1

[a] Value from: K. E. van Holde, 'Physical Biochemistry', Prentice-Hall, 1971

dry-heating process, this does not account for the much larger average molecular weight of the complex. The general trends of behaviour detected by GPC/MALLS and ultracentrifugation are the same. The slight discrepancy in the numerical values can be accounted for by noting that the light-scattering results may be affected to some extent by additional aggregated material co-existing with the protein–polysaccharide complex, whereas such material might have moved to the cell base in ultracentrifugation measurements and would therefore not have contributed to the weight-average molecular weight.

Figure 2 compares chromatographic elution profiles for the BSA–dextran complex (2:1 molar ratio) with those for the native BSA and the heat-treated BSA. We note the different position of the complex peak (lower elution volume) from that for the protein alone, both from light-scattering analysis and differential refractive index analysis. The peak shape shows that the complex is not a single species but is polydisperse. Figure 3 shows the movement of the sedimenting boundary for the BSA–dextran complex (2:1 molar ratio). The presence of a single sedimenting boundary suggests that the polydispersity is more of a quasi-continuous character than a paucidisperse one.

Sedimentation coefficients of complex, native BSA and heated BSA are also given in Table 1. From the molecular weight and the $s_{20,w}$ value, we can estimate the frictional ratio f/f_0. Assuming that the $s_{20,w}$ value measured is not too far from the infinite dilution value, the large value of f/f_0 derived for the complex is suggestive of either a highly asymmetric or a highly hydrated entity.

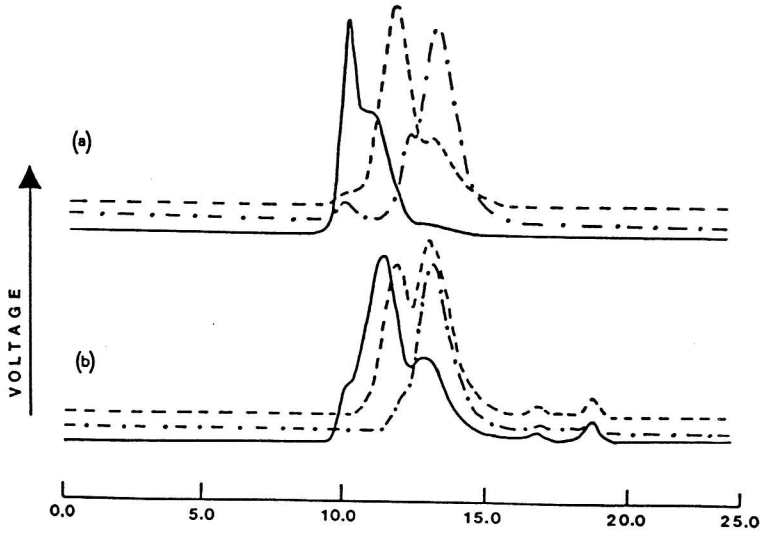

Figure 2 (*a*) *Light-scattering chromatograms (obtained from 90° detector) and* (*b*) *Differential refractive index chromatograms:* - · · · -, *native BSA;* - - -, *heat treated BSA;* —, *BSA/dextran T40* (2:1) *complex*

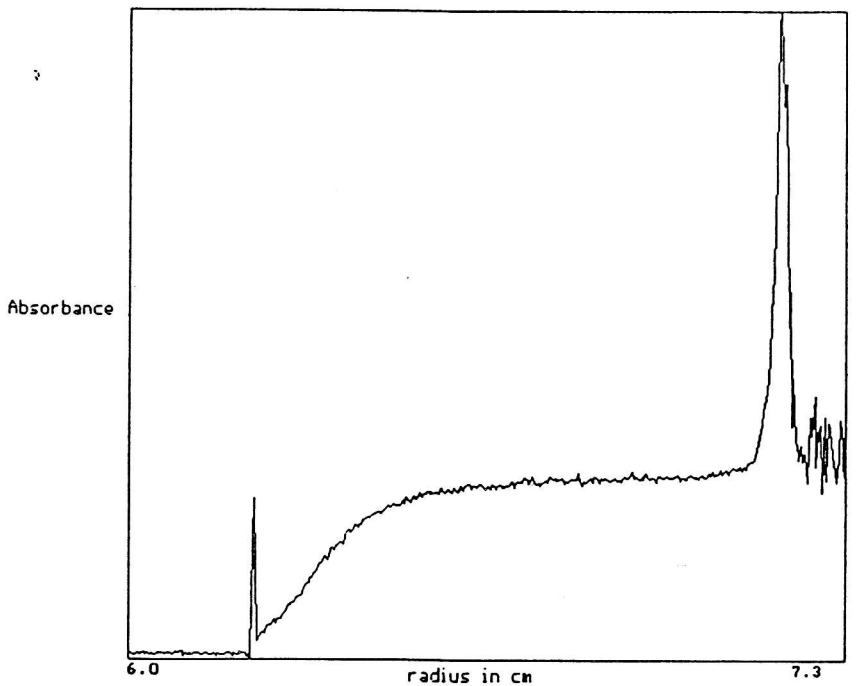

Figure 3 *Sedimenting boundary of BSA/dextran T40* (2:1) *complex*

References

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