NATURE OF PRODUCTS FORMED BY THE ACTION OF UREA ON BOVINE 
\( \beta \)-LACTOglobulins

By H. A. MCKENZIE* and G. B. RALSTON*†

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Abstract

The products of reaction of bovine \( \beta \)-lactoglobulin variants in urea at pH 3–9 were examined in two types of starch gel electrophoresis system. The first type, containing 7M urea at pH 3·5, is designed to cause minimum reversal and minimum aggregation of the products during the electrophoresis. The second type, without urea present, helps differentiate between reversible and irreversible products. The formation of irreversible products, giving rise to slower moving bands in electrophoresis, increases with increasing reaction time, urea concentration, and pH. These products are not formed when sulphhydryl reagents are present. They arise from intra- and intermolecular –SH/–SS– interchange, even at pH 3·0. After removal of urea the products were fractionated by chromatography on Sephadex G100. In the early stages of reaction a large proportion of product may be reversed to a form indistinguishable from the native protein in molecular size, mobility, and optical rotatory dispersion. Later, production of dimer (mol. wt. 36,000), trimer, tetramer, etc., in which there has been –SH/–SS– interchange, and which cannot revert completely to the native conformation, occurs. Denaturation is considered to proceed via the monomer unit.

I. INTRODUCTION

Studies have been made of the optical rotation, viscosity, and solubility changes that occur for bovine \( \beta \)-lactoglobulins A, B, and C in urea solution (Ralston 1969; McKenzie and Ralston 1971, 1973a, 1973b). In the preceding paper (McKenzie and Ralston 1973b) it was shown that the solubility changes become increasingly irreversible with prolonged reaction times. This loss of reversibility also occurs with the rotation change and is largely prevented by –SH reagents. It is likely that the “irreversible” reactions involve both intra- and intermolecular –SH/–SS– interchange and to a lesser extent –SH oxidation. The present paper describes resolution of the reaction products by two types of starch gel electrophoresis. The first, in which the gel contains urea, is designed to cause minimum reversal and minimum aggregation of products. The second, in which urea is absent from the gel, is designed to enable differentiation between reversible and irreversible products. It is shown that both types of product are formed, the latter after prolonged reaction. Reaction products have been fractionated by column chromatography and properties, such as optical rotatory dispersion (ORD) and molecular size, of some of the fractions was examined.

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II. EXPERIMENTAL

The materials used were similar to those described earlier (McKenzie and Ralston 1973b) except for the addition of β-lactoglobulin from Droughtmaster prepared by the method of Bell et al. (1970).

The methods were also similar, but with the following additions. Starch gel electrophoresis was carried out as described by McKenzie (1971) and the gels were stained with nigrosine. The following buffer systems were used:

<table>
<thead>
<tr>
<th>Buffer System</th>
<th>Gel buffer</th>
<th>Electrode buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boric acid–sodium hydroxide, pH 8.6</td>
<td>0.026 M H₃BO₃–0.01 M NaOH</td>
<td>0.3 M H₃BO₃–0.06 M NaOH</td>
</tr>
<tr>
<td>Formic acid–sodium hydroxide, pH 3.6</td>
<td>0.01 M NaOH–0.05 M HCOOH</td>
<td>0.08 M NaOH–0.16 M HCOOH</td>
</tr>
<tr>
<td>Tris–citrate–borate, pH 7.5 (Kristjansson 1963)</td>
<td>0.014 M Tris–0.004 M citric acid</td>
<td>0.3 M H₂BO₃–0.06 M NaOH</td>
</tr>
<tr>
<td>Tris–citrate–borate–lithium hydroxide, pH 7.7 (Ferguson and Wallace 1963)</td>
<td>10% (v/v) 0.02 M LiOH–0.076 M H₃BO₃</td>
<td>0.1 M LiOH–0.38 M H₃BO₃</td>
</tr>
<tr>
<td></td>
<td>90% (v/v) 0.016 M Tris–0.0033 M citric acid</td>
<td></td>
</tr>
</tbody>
</table>

Some of the gels contained 7 M urea as described in Section III.

For chromatography a glass column, 3.4 cm diam. by 40 cm, was packed with Sephadex G100 that had been allowed to swell for several days in phosphate buffer, pH 7.5, I = 0.05. The column was allowed to equilibrate in a cold room at 2±1°C, the phosphate buffer being pumped through the column at 30 ml/hr. Calibration of the elution volumes from the column in terms of the molecular weight of typical globular proteins was carried out by the method of Andrews (1964, 1965).

III. RESULTS

(a) Effect of Urea Concentration on Electrophoresis Patterns

Bovine β-lactoglobulin B (c. 10 g/l) was allowed to react with urea of 4, 5, 6, and 7 M, pH 5.2, for 24 hr at 25°C. Samples of the urea solutions were then applied to starch gels in borate buffer at pH 8.6, which contained no urea. A control sample (pH 5.2) that had not been exposed to urea was also applied, and the electrophoresis was carried out. McKenzie and Sawyer (1966) have shown that β-lactoglobulin that has not been allowed to stand at pH 8.6 prior to the electrophoresis gives rise to a single band in the borate buffer system. Such a pattern was obtained for the control sample (Fig. 1a). However, the samples that were in urea solution > 4 M gave rise to multiple bands, the fastest moving of which had identical mobility with that of the control sample. The intensity of the fast-moving band decreased with increasing urea concentration, whereas the number and intensity of the other bands increased. The occurrence of multiple bands after exposure to concentrated urea was also shown by the A, C, and Droughtmaster variants. [Droughtmaster has the same amino acid composition as A, but has a carbohydrate moiety (Bell et al. 1970).]

It appears that the first band represents a product of the reaction at pH 5.2 that is partially or completely reversed when the electrophoresis is run in a gel without added urea, whereas the slower moving bands represent irreversible products.

(b) Effect of pH of Urea Reaction on Patterns

The effect of reaction with urea at pH 3.5–8.9 on the electrophoresis patterns at pH 8.9 was then examined. Although the slow bands were produced more rapidly and extensively in solutions of higher pH, the patterns appeared to be generally
similar following reaction at pH 3.5, 5.2, 7.5, and 8.9 (see Fig. 2a). The bands appeared within 1 hr of mixing at pH 8.9 and maximum intensity was reached within 24 hr reaction. Even in the absence of urea, holding the solution at pH 8.9 for 24 hr prior to electrophoresis produced considerable material giving rise to slow-moving bands (Fig. 1b) as found earlier (McKenzie and Sawyer 1966).

(c) Effect of N-ethylmaleimide

When the reaction in urea was carried out in the presence of $1 \times 10^{-3} \text{M}$ N-ethylmaleimide, the slow-moving bands were no longer detected. Thus $\text{SH}/\text{SS}$-interchange is implicated in their formation.

(d) Effect of Presence of Urea during Electrophoresis

Solutions of bovine $\beta$-lactoglobulin B (c. 10 g/l) were allowed to react with 7M urea at pH 3.5, 5.2, 7.5, and 8.9 at 25°C for 48 hr. The samples of the solutions were then applied to gels containing 7M urea at pH 3.6 (formate buffer) and 8.9 (borate buffer). The resulting electrophoresis patterns are shown in Figures 2(c) and 2(b) respectively. A pattern of a gel at pH 8.9 containing no urea is also shown (Fig. 2a). The slow-moving bands are produced even when urea is not in the gel. The alternation in the intensity with which these bands stain is to be noted.

It can be seen from the control pattern $N$ in Figure 2(b) that, if urea is present in the gel during electrophoresis at pH 8.9, extensive further interaction occurs during the time of the electrophoresis (cf. control at pH 3.5).

The mobilities of the new bands produced after exposure to urea at pH 3.5 are identical with those of the corresponding bands produced after exposure to urea at pH 5.2 and 8.9. However, the mobilities of all the bands present in the gels containing 7M urea are less than those of the equivalent bands in the gels not containing urea.
It is important to realize that $\beta$-lactoglobulin is fully unfolded in 7M urea at pH 3.5, and hence the control pattern labelled $N$ represents protein that has been unfolded by the 7M urea in the gel. When the electrophoresis was carried out for only 6 hr it was found that the B variant gave rise to a single band with a diffuse

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**Fig. 2.—Zone electrophoresis of bovine $\beta$-lactoglobulin B after reaction in 7M urea at pH 8.9, 7.5, 5.2, and 3.5 for 24 (a) or 48 hr (b, c).** (a) Electrophoresis was at 180 V for 6 hr, in 15% starch, borate buffer, pH 8.6. (b) Electrophoresis at 180 V for 6 hr, in 15% starch, borate buffer plus 7M urea, pH 8.8. (c) Electrophoresis at 180 V for 11 hr, in 15% starch, formate buffer plus 7M urea, pH 3.6. $N$, protein sample that had not been subjected to urea denaturation prior to application to the gel. Arrows indicate origin.
trailing edge. When the electrophoresis was performed for 12 hr, this edge was resolved into two bands. These bands have been designated 2a and 2b on the system developed for numbering the bands (Table 1).

**Table 1**

**Relative mobilities of slow-moving bands of denatured B variant in starch–7M urea, pH 3.5**

<table>
<thead>
<tr>
<th>Band</th>
<th>Relative mobility</th>
<th>Band</th>
<th>Relative mobility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>5</td>
<td>0.47</td>
</tr>
<tr>
<td>2a</td>
<td>0.87*</td>
<td>6</td>
<td>0.38</td>
</tr>
<tr>
<td>2b</td>
<td>0.80</td>
<td>7</td>
<td>0.34</td>
</tr>
<tr>
<td>3a</td>
<td>0.71</td>
<td>8</td>
<td>0.29</td>
</tr>
<tr>
<td>3b</td>
<td>0.69</td>
<td>9</td>
<td>0.26</td>
</tr>
<tr>
<td>3c</td>
<td>0.65</td>
<td>10</td>
<td>0.22</td>
</tr>
<tr>
<td>4</td>
<td>0.53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Standard error from four determinations of the bands = ±0.02.

A comparison was made of the electrophoretic behaviour of the denatured A, B, and C variants in the 7M urea, pH 3.5, buffer system. It is seen in Figure 3 that they give rise to qualitatively similar patterns. Because the –SH/–SS– interchange of the unfolded protein is small during electrophoresis in this system, and it was found to give good resolution of the bands, it was used in further investigations.

![Fig. 3.—Starch gel electrophoretic patterns of bovine β-lactoglobulin A, B, and C after reaction in 7M urea at pH 3.5, 25°C, for 24 hr. N, variant A protein in 0.1M NaCl applied to the gel without reaction in urea (control). Electrophoresis in 15% starch, formate buffer plus 7M urea, pH 3.5; 150 V for 6 hr. The origin was at the base of the figure.](image)

The effect of time of exposure of the B variant to 7M urea at pH 3.5, 5.2, or 8.9 prior to electrophoresis was investigated. Electrophoresis in starch gels containing 7M urea at pH 3.5 was carried out on protein that had been denatured for 12 hr and
4 days. The longer time of reaction had little effect on the general type of pattern produced. However, band 1 was eliminated following 4 days reaction at pH 5·2, and two new bands (6, 7) appeared. The distribution of bands 2 and 3 changed with time of reaction. The intensity of the faster of these bands decreased with time while the slower bands increased in intensity.

(e) Interactions during Electrophoresis

In order to demonstrate that the multiple zones in the patterns observed did not arise from interactions during electrophoresis, a strip was cut from the unstained portion of a gel, containing 7M urea at pH 3·5, and in which a solution of B in 7M urea at pH 5·2 had been subjected to electrophoresis. This strip was inserted in a sample slot in a second, identical gel, and electrophoresis carried out at right angles to the original direction, giving rise to a diagonal pattern. No trace of protein moving ahead of, or behind, each zone was detected. Thus the zones observed in the urea-formate gel do not arise from interactions during the electrophoresis.

(f) Effect of 2-Mercaptoethanol

Protein that had been denatured in 7M urea was treated with 2-mercaptoethanol, then applied to a gel containing 7M urea, formate buffer (pH 3·5), and 2-mercaptoethanol (5 x 10^{-2}M), and electrophoresis performed. The resulting pattern showed a single, spread band.

(g) Effect of Temperature at pH 8·9

Two solutions of the B variant (9·6 g/l) in 7M urea, pH 8·9, were prepared and one was allowed to stand at 25°C and the other at 3°C. Samples were withdrawn at 1 and 48 hr, and starch gel electrophoresis in 7M urea, pH 3·5, was performed. After 1 hr at 25°C band 1 was not detected, but band 3 was prominent. On the other hand, band 1 could still be detected in the sample from the 3°C reaction, and band 2 was of greater intensity than band 3. After 48 hr there was no noticeable change in the total number of bands produced by the system at 25°C, but band 2 had become weak in intensity while band 3 had become more intense. The number of bands produced after 48 hr at 3°C was greater than the number produced after 1 hr, and the slower moving components stained more intensely than the corresponding material produced at 25°C.

(h) Chromatography on Sephadex G100

Reaction products after various times, for the protein in 7M urea, were dialysed against phosphate buffer, pH 7·5, separated by chromatography on Sephadex G100 and then characterized with respect to electrophoretic properties and ORD. An approximate estimate was also made of their molecular size. The elution profiles of samples that had been withdrawn at various times from the reaction mixture of bovine β-lactoglobulin B in 7M urea, pH 7·5, at 25°C, are shown in Figure 4. The peaks observed are designated I–IV in order of decreasing elution volume. In Table 2 the elution volumes of the peaks are compared for reaction times of 1, 24, and 48 hr. Also included are estimates of the apparent molecular weights of the fractions, as
Fig. 4.—Elution profiles of bovine \(\beta\)-lactoglobulin B chromatographed on a Sephadex G100 column after various reaction times in 7M urea, pH 7·5, 25°C; and starch gel electrophoretic patterns of total protein and fractions from the column. (a) Native protein, control; (b) after 1 hr in 7M urea; (c) after 24 hr; (d) after 48 hr. In (c) an elution profile for the C variant, after 24 hr in 7M urea, is shown by a broken line. I, II, III, IV signify the fractions shown. \(N\), B protein that had not been in contact with urea; \(D\), total protein after 24 hr reaction in urea.
determined from the elution volumes and the calibration curve for the separation column. Peaks I–IV appear to represent material of molecular weight 40,000, 60,000, 80,000, and 100,000 respectively (i.e. dimer, trimer, tetramer, and pentamer of the monomer unit of molecular weight 18,000). A similar experiment was carried out on the C variant, with similar results, as can be seen from its elution profile in Figure 4(c.)

**TABLE 2**

ELUTION VOLUMES AND APPARENT MOLECULAR WEIGHS OF PRODUCTS FROM REACTION OF B VARIANT IN 7M UREA, pH 7-5*

<table>
<thead>
<tr>
<th>Peak</th>
<th>IV</th>
<th>III</th>
<th>II</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elution volume (ml)</td>
<td>114†</td>
<td>129</td>
<td>146</td>
<td>171</td>
</tr>
<tr>
<td>Apparent mol. wt.</td>
<td>100,000</td>
<td>80,000</td>
<td>60,000</td>
<td>40,000</td>
</tr>
<tr>
<td>Denaturation time 1 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elution volume (ml)</td>
<td>111</td>
<td>132†</td>
<td>150</td>
<td>183</td>
</tr>
<tr>
<td>Apparent mol. wt.</td>
<td>120,000</td>
<td>75,000</td>
<td>57,000</td>
<td>35,000</td>
</tr>
<tr>
<td>Denaturation time 24 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elution volume (ml)</td>
<td>111</td>
<td>129†</td>
<td>152</td>
<td>183</td>
</tr>
<tr>
<td>Apparent mol. wt.</td>
<td>120,000</td>
<td>80,000</td>
<td>55,000</td>
<td>35,000</td>
</tr>
<tr>
<td>Denaturation time 48 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Native B protein had an elution volume of 173 ml and an apparent molecular weight of 39,000.
† Signifies shoulder or inflection point.

(i) Electrophoresis of Eluted Fractions

Patterns for electrophoresis of some of the chromatographic fractions in 7M urea at pH 3.5 are shown next to the elution profiles in Figure 4. Patterns for protein of peaks I and II, obtained by fractionation of the products of denaturation for 1 hr, are shown in Figure 4(b). Peak I gave a band of identical mobility with that of the control protein which had not been denatured prior to electrophoresis. Peak II gave rise to electrophoretic bands 2 and 3. There was much less peak I protein present in the reaction products after 24 hr denaturation and it gave bands in positions 1 and 2. Peak II material, obtained by fractionation of the products of reaction for 24 or 48 hr, consisted primarily of protein giving band 3. Peak IV material was poorly resolved in electrophoresis and showed some bands moving faster than the control protein. Prolonged dialysis of the products of denaturation caused a change in their electrophoretic patterns with reappearance of protein with the same mobility as the control protein.

(j) Optical Rotatory Dispersion

Samples from peak I protein that had been obtained by fractionation of the products after reaction in urea for 1 and for 24 hr, and from peak IV protein after
24 hr reaction, were adjusted to a concentration of 0·8 g/l and their ORD measured (Fig. 5). The ORD of peak I protein obtained from reaction products after 1 hr denaturation was indistinguishable from that of native protein. However, protein of peak I obtained from products of 24 hr reaction did not have the 280–300 nm Cotton effects associated with the native protein. The position of the major trough was shifted towards lower wavelength. Even greater shift in the position and magnitude of this trough was evident in the ORD of protein from peak IV, which also did not have the Cotton effects near 280 nm.

![Graph showing ORD of fractions of reaction products](image)

Fig. 5.—ORD of fractions of reaction products from bovine β-lactoglobulin B in 7 M urea, pH 7·5, 25°C. Curve I is for fraction I obtained from material produced after 24 hr reaction. Broken line represents curve for fraction I obtained from material after 1 hr denaturation (indistinguishable from native protein curve). Curve IV is for fraction IV obtained after 24 hr reaction. \([m'] = \text{reduced mean residue molecular rotation.}\)

IV. DISCUSSION

It is visualized that three main types of product may be formed in the reaction of urea with bovine β-lactoglobulin:

1. Partially or completely denatured (unfolded) protein. These products have undergone conformational change without any aggregation or change in covalent linkages. Most (or all) of these products should revert to the original native conformation on removal of urea.

2. Partially or completely denatured protein that has also undergone intramolecular covalent linkage changes (usually –SH/–SS– interchange). On removal of urea these products are irreversible in the sense that there is no reversion to the original –SH and –SS– positions but there may be partial reversion to the native conformation.
(3) Partially or completely denatured protein that has become intermolecularly covalently linked. The intermolecular linkages usually involve \(-\text{SH}/-\text{SS}-\) interchange, although \(-\text{SH}\) oxidation may also be involved. On removal of urea these products retain their respective sizes (dimer, trimer, tetramer, etc.) but may recover part of their original conformation.

There is evidence in the present studies that all of these types of products are formed to varying extents, during the action of urea on bovine \(\beta\)-lactoglobulin variants, according to the urea concentration, pH, and temperature. They have been allowed for in the general reaction scheme we have given for the behaviour of \(\beta\)-lactoglobulin in urea (McKenzie and Ralston 1973a). There is some indirect evidence for a fourth type of product, namely denatured \(\beta\)-lactoglobulin that has aggregated by non-covalent linkages. Gel formation for bovine \(\beta\)-lactoglobulin in urea takes place over a limited range of conditions at pH 5·2, but not at pH values away from the isoionic point. It has been observed previously that non-covalent aggregation, as well as aggregation involving \(-\text{SH}/-\text{SS}-\) interchange, can occur for ovalbumin in urea (Frensdorff et al. 1953; McKenzie et al. 1955, 1963) and for bovine serum albumin in urea at pH 4 (Gutter et al. 1957).

\(\textit{a})\)\ Electrophoresis

Electrophoresis of the products of the action of urea on \(\beta\)-lactoglobulin has been carried out in two types of starch gel system. In gels containing 7M urea at pH 3·5, a control sample consisting of native protein applied to the gel gives a single band, presumably of unfolded denatured protein. Denatured protein applied to the gel shows this band in the early stages of reaction. This band is partly replaced by slower moving bands as the reaction proceeds. The extent of occurrence of these bands increases with increasing urea concentration, pH, and reaction time. At higher pH values the front-moving band (1) eventually disappears. It has been shown by diagonal electrophoresis that these bands arise from true products and not interaction of protein with electrophoresis buffer constituents. On electrophoresis of the products of the urea reaction in a variety of buffer systems, without urea present, it has been found that most of these products are irreversible. Since the slow-moving products are not formed when sulphydryl reagents are present during denaturation, it is concluded that they are caused primarily by \(-\text{SH}/-\text{SS}-\) interchange. This has been confirmed at the peptide level by McKenzie et al. (1972). It will be recalled that the slow, and concentration-dependent, viscosity changes in urea at pH 8·9 were also eliminated by \(N\)-ethylmaleimide. It has been shown previously that slow-moving products are formed at pH 8·6 on standing, even in the absence of urea, and it was concluded that they arise from \(-\text{SH}/-\text{SS}-\) interchange of the protein following a conformational change (McKenzie and Sawyer 1966). On the other hand, Akroyd (1965) concluded that similar slow-moving products in acrylamide electrophoresis arise from \(-\text{SH}\) oxidation. However, irreversible products are produced in alkaline solution and in urea solution when copper(II) and oxygen are absent (Buchet et al. 1966; Johansen 1953).

Although the tendency to form slow-moving products decreases with decreasing pH, the corresponding bands at all pH values have identical mobility in a given buffer
system. Thus the irreversible products at low pH are fairly similar to those produced at higher pH.

(b) Molecular Size and Optical Rotatory Dispersion

Bovine $\beta$-lactoglobulin B was allowed to react for 1 hr in 7M urea at pH 7·5, then the products of the reaction were dialysed to remove urea and subjected to gel filtration chromatography. Material from fraction I had an apparent molecular weight of 40,000 and an ORD identical with that of the native protein at pH 7·5. Fraction I gives rise to band 1 on electrophoresis in 7M urea at pH 3·5. This material probably corresponds to protein that was unfolded and dissociated primarily to single-chain monomer (18,000 mol. wt.) in 7M urea at pH 7·5, probably without intramolecular -SH/-SS- interchange (certainly without intermolecular exchange), and then refolded, on removal of urea, and associated to form primarily dimer (mol. wt. 40,000). It unfolds and probably largely dissociates to monomer (18,000) in running on the starch gel in 7M urea at pH 3·5. Its mobility corresponds to the native control sample which is also unfolded and probably largely dissociated to monomer in the gel.

After 24 hr reaction in 7M urea at pH 7·5, the products of reaction show virtually no band 1 on electrophoresis in 7M urea at pH 3·5, but some band 2b is present. When the urea is removed from the reaction mixture which is then subjected to gel filtration, a fraction of apparent molecular weight 40,000 (dimer) is obtained. This material gives an ORD curve that is broadly similar to that of the native protein, but the Cotton effects in the 280 nm region are absent. It is suggested that this product arises from unfolding of the $\beta$-lactoglobulin and inter- or intramolecular interchange or both during the reaction in urea. A band, corresponding to band 2 in 7M urea gels, is not observed in gels that do not contain urea. In the latter gels the native protein band probably represents a mixture of monomer and dimer, with dimer predominating. “Band 2”-type protein would probably not be resolved from the native protein band in these gels.

Material from the Sephadex column chromatography with an apparent molecular weight of 60,000 (trimer) gives rise to band 3 (with a trace of band 2) on electrophoresis in 7M urea at pH 3·5. This material is an important component in the reaction products eluted from the Sephadex column, while that of molecular weight 80,000 appears only as an inflection point or shoulder on the larger peak which contains all the material of molecular weight greater than 100,000.

Subcomponents that are observed in bands 2 and 3 may represent species of the same molecular size, but having different arrangements of the -SS- bridges. The slower moving band in each group may be those in which intramolecular rearrangement has occurred. The relative increase in the slower subcomponents with time is in accord with this hypothesis. All this evidence supports our contention that denaturation proceeds via the monomer unit.

The electrophoretic bands moving slower than band 3 presumably represent higher polymers. The ORD of such material on removal of urea has a broad similarity to the ORD of the native protein. The trough near 230 nm is shifted towards 226 nm, and the magnitude of the rotation is twice that of the native protein. The Cotton
effects near 280 nm are missing. These aggregates have partly refolded on removal of urea, but probably contain appreciable cross β-structure (cf. Astbury et al. 1935).

The conclusions reached in this paper on the irreversible products of the action of urea on bovine β-lactoglobulin are contrary to those of Morr (1967). He concluded that only the physical properties of β-lactoglobulin are altered and that any -SH/-SS-interchange is intra- and not intermolecular. Although he observed slow-moving bands on acrylamide gel electrophoresis, he only observed broadening of the β-lactoglobulin peak on Sephadex G100. Morr applied a 5-ml sample to a Sephadex column 1·5 by 25 cm (void volume 10 ml). We have calculated that on such a column our components I-IV would have elution volumes of 18·9, 16·1, 12·7, and 11·7 ml respectively. Thus with a 5-ml sample volume it is not surprising that he did not achieve resolution on his column. We have checked for the presence of bands of the type found by Cejka et al. (1968), formed by carbamylation of the protein due to cyanate in the urea. Such bands were not present in our experiments carried out with protein in urea for 4 days.

V. ACKNOWLEDGMENT

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VI. REFERENCES


