Valyl-tRNA Synthetase from Rabbit Liver

I. PURIFICATION AS A HETEROPTIC COMPLEX IN ASSOCIATION WITH ELONGATION FACTOR 1*

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Valyl-tRNA synthetase occurs as a high molecular mass entity of ≈ 700 kDa in the crude extract from rabbit liver. The enzyme was purified as a heterotypic complex comprising four polypeptides of 140, 50, 35, and 27 kDa in the molar proportions of 1:2:1:1, respectively, as determined by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Co-purification of these components at each step of the purification supports the conclusion that they are physically associated within the same complex. In addition to valyl-tRNA synthetase activity, which was assigned to the 140-kDa component, the purified complex exhibits a potent Elongation Factor 1 activity, determined by its ability to sustain poly(U)-dependent polyphenylalanine synthesis in the presence of Elongation Factor 2. Our results are essentially in agreement with those from a recent report (Motorin, Y., Wolfson, A., Orlovsky, A., and Gladilin, K. (1988) FEBS Lett. 238, 262–264) according to which the polypeptides other than that assigned to valyl-tRNA synthetase correspond to the subunits of Elongation Factor 1H.

Unlike the valyl-tRNA synthetases from prokaryotes and lower eukaryotes, which are monomeric enzymes of 110 and 125 kDa, respectively, that from various mammalian cell lines occurs as a high-M₉ entity (1, 2). By gel filtration on Bio-Gel A-5m, the enzyme from the crude extract of Chinese hamster ovary cells was previously shown to elute as a complex of apparent M₉ 700,000 (3), clearly distinguishable from a larger complex of molecular weight 1.2 × 10⁶, containing the nine aminoacyl-tRNA synthetases specific for isoleucine, leucine, methionine, glutamic acid, glutamine, lysine, arginine, aspartic acid, and proline. Moreover, none of the remaining 10 aminoacyl-tRNA synthetases co-eluted with valyl-tRNA synthetase, implying that this high-M₉ entity was either a homotypic multimer of valyl-tRNA synthetase or else a heterotypic association of this enzyme with components other than aminoacyl-tRNA synthetases. Two recent studies have addressed this issue with conflicting results. According to one report (4), the valyl-tRNA synthetase complex from rat liver is a homotypic tetramer with subunits of 140 kDa, while others (5) have reported that the enzyme purified from rabbit liver is a heterotypic complex composed of several polypeptides, with molecular masses of 130, 50, 40, and 30 kDa, respectively. Furthermore, in a more recent contribution from the same laboratory (6), the polypeptides other than that of 130 kDa were assigned to the subunits of Elongation Factor 1H.

Previous purifications of valyl-tRNA synthetase from rat (4) and rabbit (5) livers were conducted on a small scale, starting from about 50 g of tissue. Moreover, in the latter study, the yields at the various steps of the purification were not specified. The aim of our study was to attempt to resolve the discrepancy between these published results and to develop a larger scale purification of the valyl-tRNA synthetase complex in order to obtain adequate supplies of this material for further characterization. This paper reports the purification to apparent homogeneity of the valyl-tRNA synthetase complex, starting from 1 kg of rabbit liver. The evidence presented supports the view that the purified heterotypic complex is representative of the high-M₉ form of valyl-tRNA synthetase present in the crude extract. The properties of the purified complex are essentially in agreement with those reported by Motorin et al. (5, 6), in regard to both its polypeptide composition and the presence of a potent Elongation Factor 1 activity.

EXPERIMENTAL PROCEDURES†

RESULTS

Purification of Valyl-tRNA Synthetase—Table I summarizes the purification of valyl-tRNA synthetase from rabbit liver, conducted as described under "Experimental Procedures." The enzyme was purified 3600-fold as a heterotypic complex, with an overall yield of 10% and a specific activity of 252 units mg⁻¹ at 25 °C.

The size distribution of valyl-tRNA synthetase in the crude extract was examined by subjecting an aliquot to gel filtration on Sepharose 6B. The major portion of the activity was eluted as a fairly broad peak displaying a mean apparent M₉ of about 700,000, while approximately 10% was located in the void volume, presumably reflecting association to components of the microsomal fraction (result not shown).

Fractionation of the crude extract with ammonium sulfate from 34 to 42% saturation resulted in 4-fold purification of the enzyme, with a yield close to 60%. This narrow precipitation range afforded the best compromise between yield and extent of purification. Fractional precipitation with polyethylene glycol 6000 was less effective. Whereas over 85% of the multi-aminoacyl-tRNA synthetase complex of M₉ 1.2 × 10⁶ was precipitated between 2 and 5% polyethylene glycol (2), less than 50% of the valyl-tRNA synthetase activity was recovered in this fraction, the remainder precipitating be-

† Portions of this paper (including "Experimental Procedures," Figs. M1–M3, and Tables M1 and M2) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
tween 0.5 and 2%. This suggests that polyethylene glycol may promote the self-aggregation of the complex or its interaction with other cellular components through exclusion effects.

A 20-fold purification of valyl-tRNA synthetase was achieved by subjecting the ammonium sulfate fraction to preparative gel filtration. The activity was recovered as a single, broad peak corresponding to a mean apparent M₆, similar to that found in the crude extract (Fig. M1A). Further purification was accomplished by successive chromatographic runs on phosphocellulose, hydroxylapatite, DEAE-cellulose, and heparin-Ultrogel. At each of these steps, the flow-through fractions were devoid of valyl-tRNA synthetase activity. High recovery of enzyme activity as a single yet fairly broad peak was obtained from phosphocellulose and hydroxylapatite, using linear salt gradients (Fig. M1, B and C). The phosphate concentrations required to elute the activity were about 130 and 180 mM, respectively. At the subsequent DEAE-cellulose chromatographic step, valyl-tRNA synthetase was recovered as two partially overlapping peaks, eluted at about 100 and 200 mM KCl, respectively (Fig. M1D). The polypeptide composition of the fractions containing activity was examined by subjecting aliquots to SDS-polyacrylamide gel electrophoresis (Figs. 2 and M2). The major activity peak II (fractions 18–30) contained mainly five polypeptide components, only four of which (140, 50, 35, and 27 kDa) were present in the same relative proportions throughout the peak. These same four components were also present under the minor peak I (fractions 9–15), together with several additional components.

The total recovery of valyl-tRNA synthetase activity from DEAE-cellulose was 80%. Fractions 18–27, containing 54% of the input activity, were combined for further purification on heparin-Ultrogel. Enzyme activity was recovered as a single, symmetrical peak eluting at about 100 mM potassium phosphate (Fig. 1). Moreover, the four polypeptides of 140, 50, 35, and 27 kDa were present in the same relative proportions in each of the active fractions. Compared to the material from the DEAE peak, that recovered after heparin-Ultrogel chromatography displayed a 10% gain in specific activity, compatible with the elimination of the minor components that were unevenly distributed under the DEAE-cellulose peak II.

Taken together, these results supported the view that valyl-tRNA synthetase was purified to apparent homogeneity as a heterotopic complex composed of four polypeptides. This was confirmed by subjecting the purified complex to gel filtration on TSK 3000. As shown in Fig. M3, the single valyl-tRNA synthetase activity peak was coincident with that of thyroglobulin (M₆, 669,000) used as a marker in a separate run.

Moreover, the activity peak contained each of the four polypeptides that were present in the heparin-Ultrogel fraction (result not shown).

It is noteworthy that polypeptides displaying electrophoretic mobilities corresponding to those present in the purified complex were present in the combined active fractions after each of the chromatographic steps (Fig. 2). This result, together with the finding that valyl-tRNA synthetase activity was recovered as a high-M₆ entity after each step of the purification, support the conclusion that the purified complex is representative of the high-M₆ entity present in the crude extract.

Valyl-tRNA synthetase activity was remarkably stable throughout the purification steps. The overall yield of the five chromatographic steps, starting from the ammonium sulfate fraction, was 17%. However, taking into account the leading and tailing portions of the peaks left out at each step, approximately 50% of the input activity was accounted for. Moreover, the purified complex remained fully active for at least 5 months when stored at −20 °C in the presence of 50% glycerol.

**Structural Properties of the Complex**—The valyl-tRNA synthetase complex purified to homogeneity is composed of four polypeptides with apparent molecular masses of 140, 50, 35, and 27 kDa, as visualized by one-dimensional SDS-polyacrylamide gel electrophoresis (Fig. 2). Their relative molar ratios were estimated to be 1:2:1:1, respectively, by densitometric scanning of Coomassie-stained gels. The minimal molecular mass of the complex calculated from these values corresponds to about 300 kDa, suggesting that the mean apparent M₆ of 700,000 estimated by gel filtration reflects self-association of

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**TABLE 1**

**Purification of valyl-tRNA synthetase from rabbit liver**

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg)</th>
<th>Yield (%)</th>
<th>Purification (fold)</th>
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<tr>
<td>Crude extract</td>
<td>95.880</td>
<td>6,723</td>
<td>0.07</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>13,225</td>
<td>3,900</td>
<td>0.3</td>
<td>58</td>
<td>4</td>
</tr>
<tr>
<td>precipitate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Bio-Gel A-5m</td>
<td>433</td>
<td>2,552</td>
<td>5.9</td>
<td>38</td>
<td>84</td>
</tr>
<tr>
<td>Phosphocellulose</td>
<td>139</td>
<td>2,271</td>
<td>16</td>
<td>34</td>
<td>233</td>
</tr>
<tr>
<td>Hydroxylapatite</td>
<td>35.2</td>
<td>1,635</td>
<td>46</td>
<td>24</td>
<td>664</td>
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<tr>
<td>DEAE-cellulose</td>
<td>3.6</td>
<td>850</td>
<td>236</td>
<td>13</td>
<td>3,373</td>
</tr>
<tr>
<td>Heparin-Ultrogel</td>
<td>2.6</td>
<td>654</td>
<td>252</td>
<td>10</td>
<td>3,600</td>
</tr>
</tbody>
</table>

The abbreviations used are: SDS, sodium dodecyl sulfate; EF, elongation factor.
The underlying assumption that the activity of valyl-tRNA synthetase is not significantly influenced by its association to the complex is supported by the results presented in the accompanying paper (8). This value is within the range of those found for three dimeric aminoacyl-tRNA synthetases having similar molecular masses (about 150 kDa), purified to homogeneity from the same source: threonyl-tRNA synthetase 485 units mg\(^{-1}\) at 25 °C, seryl-tRNA synthetase 400 units mg\(^{-1}\), and histidyl-tRNA synthetase 600 units mg\(^{-1}\).3

Based on the apparent molecular masses of 110 and 125 kDa for the monomeric valyl-tRNA synthetases from Escherichia coli (9) and yeast (10), respectively, it was anticipated that the 140-kDa polypeptide of the complex corresponds to valyl-tRNA synthetase. This assignment was verified by the finding that antibodies raised against valyl-tRNA synthetase from yeast specifically recognized the 140-kDa component of the complex by the immunoblotting procedure (result not shown). It was subsequently confirmed by showing that the valyl-tRNA synthetase dissociated from the complex and purified to homogeneity co-migrates with the 140-kDa component of the complex by SDS-polyacrylamide gel electrophoresis (8).

According to Motorin et al. (6), the other polypeptide components of the complex correspond to the subunits of Elongation Factor 1H. The presence of EF-1 activity in our purified complex was established by assaying for poly(U)-dependent polyphenylalanine synthesis. As shown in Table M1, the assay system used was dependent on the addition of EF-1α as well as EF-2. Moreover, the purified complex could effectively substitute for EF-1α but not for EF-2. The initial rate measurements presented in Fig. 4 show that EF-1α contained in the purified complex was significantly more active than the homogenous EF-1α from calf brain. The corresponding specific activities were, respectively, 582 and 157 nmol of p-nitroanilide min\(^{-1}\) mg\(^{-1}\) and 1156 and 457 nmol of p-nitroanilide min\(^{-1}\) mg\(^{-1}\), respectively, as visualized by one-dimensional SDS-polyacrylamide gel electrophoresis (11).

The EF-1α activity of the purified complex was assigned to the 50-kDa polypeptide, based on its co-migration with EF-1α from calf brain on SDS-polyacrylamide gels (Fig. 2).

**DISCUSSION**

In the present study, valyl-tRNA synthetase from rabbit liver was purified 3600-fold to apparent homogeneity, as a heterotypic high-M₉ complex. The evidence presented supports the view that the four polypeptides of 140, 50, 35, and 27 kDa, that are present in the molar ratios of about 1:2:2:1, respectively, as visualized by one-dimensional SDS-polyacrylamide gel electrophoresis, are physically associated within the same complex. This polypeptide composition is essentially in agreement with that reported recently for the complex from the same source purified on a small scale using high resolution chromatographic procedures (5).

The valyl-tRNA synthetase purified from rat liver by Godard and Yang (4) differs from that described in this paper by the following properties: (i) one-dimensional SDS-polyacrylamide gel electrophoresis displayed a single polypeptide located between the positions of the isoleucyl- and leucyl-tRNA synthetase components of the multienzyme complex from rat liver used as a size marker, whereas the polypeptide which we assigned to valyl-tRNA synthetase in our purified complex migrates slightly behind the polypeptide corresponding to isoleucyl-tRNA synthetase from rabbit liver (Fig. 2). This difference in migration of the two valyl-tRNA synthetases relative to the isoleucyl-tRNA synthetase marker is significant, since we previously showed that the isoleucyl-tRNA synthetase component of the complexes from rat and rabbit livers have identical electrophoretic mobilities (12); (ii) the valyl-tRNA synthetase purified from rat liver was re-

3 G. Gangloff and J. P. Waller, unpublished results.
Mammalian Valyl-tRNA Synthetase Complex

Fig. 3. Electron micrograph of the purified valyl-tRNA synthetase complex. The specimen prepared as described under “Experimental Procedures” was negatively stained with phosphotungstic acid adjusted to pH 6.5. Bar = 100 nm.

Fig. 4. Poly(U)-dependent polyphenylalanine synthesis catalyzed by EF-1α from calf brain (■) or by the purified valyl-tRNA synthetase complex from rabbit liver (■). Polyphenylalanine synthesis was measured as described under “Experimental Procedures,” using an assay system that was dependent on the addition of EF-1α. The molar concentrations of added valyl-tRNA synthetase complex and EF-1α were based on molecular weights of 300,000 and 50,000, respectively, which correspond to equal inputs of EF-1α.

ported to be extremely labile, in contrast to the pronounced stability found in the present study; (iii) at moderate salt concentration, the purified native enzyme from rat liver behaved as a monomeric entity of Mw 140,000 by gel filtration on Sephadex G-200. However, in the presence of 400 mM KCl, an unspecified fraction of the activity was found near the void volume of the column, which the authors assimilated to the high-Mw form originally present in the crude extract (4).

A plausible explanation to account for these differences is that valyl-tRNA synthetase from rat liver underwent limited proteolysis in the course of its purification, yielding an active, yet labile, modified enzyme no longer able to form the heterotypic complex described in the present study.

In a more recent report on the valyl-tRNA synthetase complex from rabbit liver, the polypeptides other than that of 140 kDa which corresponds to the enzyme, were assigned to the subunits of EF-1H (6). The evidence was based on the similarity of the polypeptide patterns afforded by the purified EF-1H from rabbit reticulocytes and the valyl-tRNA synthetase complex from rabbit liver by two-dimensional electrophoresis, as well as on the finding that these two complexes displayed comparable EF-1 activities in poly(U)-dependent polyphenylalanine synthesis.

The properties of the purified valyl-tRNA synthetase complex described in the present study are in agreement with this assignment. Not only does this complex effectively catalyze poly(U)-dependent polyphenylalanine synthesis in the absence of added EF-1α, but the significantly higher rate of synthesis compared to that obtained in the presence of molar equivalents of homogenous EF-1α, is best accounted for by assuming the presence of the complementary factors EF-1βγ, which facilitate the GDP-GTP exchange reaction (see below). Moreover, the 2:1 molar stoichiometry displayed by the 50-kDa, 35-kDa and 27-kDa subunits of the valyl-tRNA synthetase complex after one-dimensional SDS-polyacrylamide gel electrophoresis, is in accord with the established fact that two of the subunits of EF-1H, namely EF-1α and EF-1γ, possess the same molecular mass of 50 kDa. The existence of two distinct 50-kDa components in the valyl-tRNA synthetase complex was confirmed by the results presented in the accompanying paper (8).

It is now well established that in the crude extracts from eukaryotic cells, a fraction of the Elongation Factor 1α occurs as a complex designated as EF-1H, that comprises the complementary factors EF-1β and -γ (13). EF-1α (Mw 50,000, pI 9) and EF-1β (Mw 26,000–30,000, pI ≈ 5.5) are functionally equivalent to bacterial EF-Tu and EF-Ts, respectively. EF-1γ (Mw 50,000, pI ≈ 6), the precise fraction of which has yet to be established, acts in concert with EF-1β to facilitate the exchange of EF-1α-bound GDP to GTP. The functional properties of EF-1H in relation to protein synthesis have been extensively studied (13). The prevailing view is that GTP-induced dissociation of the complex to EF-1α-GTP and EF-1βγ is a prerequisite for the formation of the functional ternary complex EF-1α-GTP-aminocyl-tRNA (14). It has also been shown that dissociation of the binary complex EF-1βγ requires denaturing conditions, such as the presence of 6 M urea (15–17). That hydrophobic interactions are involved in the βγ association is supported by the pronounced hydrophobic character of EF-1γ from Artemia salina (15, 18), as well as the tendency of EF-1βγ from most sources to form high-Mw aggregates in vitro (17, 19).

Depending on the eukaryotic species examined, EF-1H was purified either as a homodisperse entity containing one mole each of the α, β, and γ subunits, or as polydisperse aggregates of Mw > 500,000 containing equimolar amounts of the three subunits (Table M2), as is the case for EF-1H from rabbit reticulocytes (19). The latter observation raises the possibility...
that the tendency of the valyl-tRNA synthetase complex from rabbit liver to self-aggregate is conferred by its EF-1H moiety.

Although the two-dimensional electrophoretic pattern displayed by the subunits from the rabbit reticulocyte EF-1H complex and the previously purified valyl-tRNA synthetase complex from rabbit liver were shown to be qualitatively similar (6), a closer comparison of the structural properties reported for EF-1H from rabbit reticulocytes (19) with those of the valyl-tRNA synthetase complex described in this paper reveals significant differences. Unlike the EF-1H purified from most other sources, that from rabbit reticulocytes was found to be composed of four subunits, corresponding to EF-1α (52 kDa, pI 9.2), EF-1β (38 kDa, pI 5.5), EF-1β' (33 kDa, pI 4.8), and EF-1γ (48 kDa, pI 5.9). The similarity of the amino acid compositions of EF-1β and -1β' and the molar ratio of αββ'-γ in EF-1H of about 1:0.5:0.5:1, have led to the conclusion that EF-1β' was probably a breakdown product of EF-1β, and that the native form of the complex likely contains only the α, β, and γ subunits (19). It is noteworthy that the molecular mass of 38 kDa attributed to EF-1β is considerably higher than the value of 26-30 kDa reported for EF-1β from other species (Table M2).

In the valyl-tRNA synthetase complex from rabbit liver, the subunits other than that of valyl-tRNA synthetase, display molecular masses of 50, 35, and 27 kDa and are present in the molar ratios of about 2:1:1, respectively. The existence of two distinct 50-kDa components, one of which was identified as EF-1α and the other of which most likely corresponds to EF-1γ, is supported by the data presented in the accompanying paper (8). On the other hand, the presence of 35- and 27-kDa polypeptides as integral components of the complex, as was found in three separate preparations of the complex, does not support the view that the 27-kDa subunit is a breakdown product of the 35-kDa subunit. Thus, the relationship between the ββ'-β subunits (38 and 33 kDa) found in the complex from rabbit reticulocytes and the 35- and 27-kDa subunits of the valyl-tRNA synthetase complex from rabbit liver, remains to be established. This issue is addressed further in the accompanying paper (8), in connection with the properties of the subunits dissociated from the complex.

EF-1α represents about 5% of the cytoplasmic proteins in a variety of mammalian cell lines, as determined by radioimmunassay (25). The proportion of this factor engaged in the EF-1H complex as fully active monomeric enzymes of 139 and 129 kDa, respectively, displayed markedly hydrophobic properties. The finding that the corresponding enzymes from yeast, which occur as "free" monomeric enzymes of ≈125 kDa, did not manifest this property, supported the view that hydrophobic interactions are involved in the association of the two mammalian enzymes within the multienzyme complex. The results reported in the accompanying paper (8) demonstrate that mammalian valyl-tRNA synthetase has also acquired hydrophobic properties, most likely responsible for its association with EF-1H.

Acknowledgments—We thank Jean Laporte for performing the electron microscopic analysis, Drs. A. Parmeggiani and B. Crecchet for kindly providing the purified elongation factors and ribosomes, and Dr. F. Fasio for his gift of yeast valyl-tRNA synthetase antibodies. The excellent technical assistance of M. Triconnet during part of this work is gratefully acknowledged.

REFERENCES

Mammalian Valyl-tRNA Synthetase Complex


SUPPLEMENTAL MATERIAL TO

VAlYL-tRNA SYNTHETASE FROM RAMB BIT LIVER. I. Purification as a hexameric complex associated with EF-2 by G. Crechet, D. Cancell, V. Bocchini, and A. Parmeggiani

EXPERIMENTAL PROCEDURES

Mammalian tRNA synthetases are hexamers. Depending on the state of purification the tRNA synthetase activity of EF-2 was found to be higher when assayed in the presence of 5 mM MgCl2. The tRNA synthetase activity of EF-2 was determined by measuring the incorporation of 35S-labeled tRNA into proteins in a cell free system. tRNA synthetase activity of EF-2 is sensitive to the presence of 5 mM MgCl2.

Table M 2

<table>
<thead>
<tr>
<th>Structure parameters of eukaryotic elongation factor 1H</th>
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</thead>
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<tr>
<td>Pig (10)</td>
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<tr>
<td>Ammonia (15, 22)</td>
</tr>
<tr>
<td>Yeast (22, 23)</td>
</tr>
</tbody>
</table>


Fig. M1. Chromatographic elution patterns of valyl-tRNA synthetase at different stages of the purification. Chromatographic runs were carried out as described in "Experimental Procedure" (A) Bio-Gel A 5 m; (B) phosphocellulose F11; (C) hyaluronate and (D) DEAE-cellulose DE-52. Fractions combined for further purification are indicated by horizontal bars.
Fig. M2. Polypeptide composition of the valyl-tRNA synthetase-containing fractions eluted from DEAE-cellulose. Aliquots of 0.017 ml from various fractions of the DEAE chromogram shown in Fig. M1D were treated with SDS and submitted to SDS-polyacrylamide gel electrophoresis, followed by staining with silver nitrate. The fractions subjected to analysis are indicated at the top of each lane. Lanes I and II correspond to samples of the combined fractions 9 to 15 and 18 to 27, respectively. The four polypeptides corresponding to those present in the purified complex are indicated by arrows.

Fig. M3. Analytical gel filtration on TSK-3000 of the purified valyl-tRNA synthetase complex. The purified valyl-tRNA synthetase complex (6 units in 0.1 ml) was applied on a column of TSK-G 3000 SW (7.5 x 30 cm) equilibrated with 0.2 M potassium phosphate, pH 7.0, 0.5 mM dithioerythritol and 10% glycerol, at a flow rate of 0.2 ml/min. Fractions of 0.2 ml were collected and assayed for valyl-tRNA synthetase activity (++). The elution profiles of thyroglobulin (Mₚ 669,000, peak I) and of the dimeric (Mₚ 134,000, peak II) and monomeric (Mₚ 67,000, peak III) forms of bovine serum albumin were determined in a separate run by absorbance measurements at 228 nm.