The Similarity of the Two High-Molecular-Weight Polypeptides of Erythrocyte Spectrin

By MICHAEL J. DUNN,*† RICHARD B. KEMP† and ALUN H. MADDY*

*Department of Zoology, University of Edinburgh, Edinburgh EH9 3JT, Scotland, U.K., and

†Department of Zoology, University College of Wales, Aberystwyth SY23 3DA, Wales, U.K.

(Received 2 November 1977)

1. The two major polypeptides (P1 and P2) of erythrocyte-membrane spectrin were isolated by preparative polyacrylamide-gel electrophoresis. 2. The two polypeptides were shown to possess similar amino acid compositions, both with the characteristically high glutamate and leucine contents of the parent spectrin. 3. The tryptic-peptide 'maps' of the two polypeptides were prepared by a combination of t.l.c. and electrophoresis. 4. Radioactive peptides were prepared by [¹⁴C]carboxymethylation and chloramine-T-catalysed [¹²⁵I]iodination. 5. 'Maps' of both sets of peptides demonstrate a marked similarity between the two polypeptides. 6. These new data confirm earlier evidence for the similarity of the two chains. 7. The number of peptides in the 'maps' of carboxymethylated peptides suggest that polypeptides P1 and P2 are not aggregates.

Spectrin was originally considered to be a single protein, which could be extracted from erythrocyte membranes by dilute solutions of EDTA (Marchesi & Steers, 1968). Now that the extract is known to contain more than one polypeptide (Steck, 1974), the term 'spectrin' is ambiguous. The major polypeptides are two bands located in the 210 000–240 000-mol.wt. regions of a SDS/polyacrylamide-gel-electrophoresis system [P1 and P2 in the terminology of Fairbanks *et al.* (1971)] and a weaker band of mol.wt. 43 000 (P5), which has been identified as actin (Tilney & Detmers, 1975). Minor bands are also sometimes extracted, especially one (P2.1) close to band P2. Some workers, e.g. Elgsaeter *et al.* (1976), restrict the term 'spectrin' to the major components of the extract other than the actin, i.e. peptides P1+P2. We shall use the term in its original sense, i.e. for the proteins extracted from mammalian erythrocyte 'ghosts' at low ionic strength by dilute solutions of EDTA, but without any implications of homogeneity; peptides P1, P2 and P2.1 will be referred to as the 'high-molecular-weight proteins' and actin and any associated peptides as 'the low-molecular-weight proteins'.

Except for the actin, the nature of the proteins that make up the extract is obscure. We report here on the characterization of peptides P1 and P2 by amino acid composition and by peptide 'mapping'. The data suggests that the two polypeptides have many shared amino acid sequences, a conclusion which is consistent with their immunological cross-reactivity (Bjerrum *et al.*, 1975) and the similarity of

their *N*-terminal amino acid contents (Knufermann *et al.*, 1973; Maddy & Dunn, 1974; Fuller *et al.*, 1974; Dunn *et al.*, 1975). Peptide P2 cannot be derived from peptide P1 by simple amino acid deletion, for it possesses its own unique peptides, and is also distinguished from peptide P1 by being preferentially phosphorylated by membrane protein kinase (Guthrow *et al.*, 1972; Shapiro & Marchesi, 1977).

Experimental

Preparation of 'ghosts'

Fresh ox (*Bos taurus*) blood was washed three times by centrifugation (2000g, 15 min) with 0.9% NaCl to remove leucocytes and serum. The erythrocytes were haemolysed in 5 vol. of 5.0M-sodium phosphate buffer, pH 6.0, and the 'ghosts' washed repeatedly in this buffer until the supernatant was virtually free of haemoglobin. The remaining haemoglobin was removed from the 'ghosts' by washing repeatedly by centrifugation (18 000g, 20 min) with 5.0mM-sodium phosphate buffer, pH 7.5. These 'ghosts' had a slight pinkish colour, but the final trace of haemoglobin could be removed by freezing the 'ghosts' at –20°C overnight, and washing once more in the buffer at pH 7.5 (Maddy, 1966).

EDTA extraction

'Ghosts' prepared as described above were pelleted by centrifugation at 18 000g for 20 min and extracted by addition of 4 vol. of 0.5mM-EDTA, adjusting the suspension to pH 7.5 with NaOH and stirring gently for 1.5 h at 18°C.

Abbreviation used: SDS, sodium dodecyl sulphate.

† Present address: Department of Microbiology, Guy's Hospital Medical School, London SE1 9RT, U.K.

Polyacrylamide-gel electrophoresis

Analytical SDS/polyacrylamide-gel electrophoresis was carried out in the buffer system of Fairbanks *et al.* (1971). The gels, containing 7% (w/v) acrylamide and 0.1% bisacrylamide, were polymerized with ammonium persulphate and *NNN'*-tetramethylethylenediamine; they were cast in glass tubes with an internal diameter of 4 mm and each gel was 7 cm long. Protein samples were prepared by addition of dithioerythritol to 20 mM, SDS to 5%, heating at 100°C for 5 min and finally by addition of urea to 8.0 M. The gels were electrophoresed for 2 h at 5 mA per gel (10 V/cm).

Preparative electrophoresis was performed in a vertical slab gel (20 cm × 20 cm × 0.5 cm) with the same buffer, which was continuously recirculated between the two buffer chambers. An adequate separation of peptides P1 and P2 was achieved after 72 h at 7 V/cm.

Proteins were recovered from the gel by cutting the gel slab into 0.5 cm horizontal strips and macerating each strip by extrusion through a hypodermic syringe into 10 ml of 5 mM-NaHCO₃/0.5 mM-EDTA/0.05% SDS. After 16 h the gel was removed by filtration on a stainless-steel sieve and the extract centrifuged at 100 000g for 1 h to remove small pieces of gel. A second 16 h extraction with 5 ml of the same bicarbonate medium yielded a second batch of protein indistinguishable from the first. With radioactive proteins it was found that 70% of the protein in a gel was recovered by the two extractions.

Amino acid analysis

The protein samples were freeze-dried and hydrolysed in redistilled 6 M-HCl *in vacuo* at 105°C for 24, 48 or 72 h. The hydrolysates were then dried by rotary evaporation and made up to a suitable volume with 0.1 M-HCl. A 1 ml sample, to which 0.5 μmol of norleucine had been added, was fractionated on the column (type A resin) of a Technicon NC-1 amino acid analyser in which the hydriindantin was generated *in situ* by using 2 mM-hydrazine sulphate. The values were calculated after extrapolation to zero time of the results from each set of three hydrolyses.

Methionine and cysteine were measured as the sulphone and cysteic acid respectively after oxidation of protein with performic acid at 0°C (Hirs, 1957). Protein (2 mg) was mixed with 0.05 ml of 99% (v/v) formic acid and 0.01 ml of methanol and left for 30 min at 0°C. Freshly prepared cold performic acid (0.1 ml) was then added and the protein incubated for a further 2.5 h. After dilution with cold water the protein was freeze-dried and finally hydrolysed as above.

Total nitrogen was measured by a micro-Kjeldahl method (Tristram, 1966) and total amino N in a stan-

dard hydrolysate by the ninhydrin method with 5 mM-SnCl₂ as condensing agent (Holy, 1966).

*N*⁵-Methylhistidine was separated from histidine by using a 1 ml sample of standard hydrolysate made up in 0.2 M-sodium citrate buffer, pH 2.2, by elution from a normal column pre-equilibrated with 0.35 M-sodium citrate buffer, pH 5.28 (Johnson *et al.*, 1967).

Carboxymethylation

The method was adapted from that of Harris & Perham (1965). The protein sample (10 ml of EDTA extract at 1 mg/ml) was dialysed overnight against 50 mM-Tris/HCl, pH 8.0, at 4°C. Then 50 μCi of iodo-[2-¹⁴C]acetic acid (10–25 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) was dissolved in 0.5 ml of water and added to the protein (12 μCi was used for each protein fraction obtained by preparative gel electrophoresis). After 30 min at 18°C, solid urea was added to 8.0 M and, after a further 90 min, 0.1 ml of β-mercaptoethanol. After a further 60 min, the solution was dialysed at 4°C for 24 h against three changes of 50 mM-Tris/HCl, pH 8.0, to remove radioactive material of low molecular weight. Finally 30 mg of unlabelled iodoacetic acid in 0.5 ml of water (10 mg for the smaller fractions from slab electrophoresis) was added, followed by urea to 8.0 M and dithioerythritol to 20 mM. After 90 min at 18°C the mixture was dialysed overnight against the buffer used for trypsin treatment.

Purification of the [¹⁴C]carboxymethylated peptides

The peptides were concentrated to 1.0 ml by rotary flash evaporation and passed through a Sephadex G-50 column (10 cm × 2.5 cm) equilibrated with water. The column eluate was monitored at 280 nm. Radioactivity was confined to a retarded component, which was collected, acidified by the addition of formic acid to 0.1 M and applied to a SE (sulphoxyethyl) Sephadex column (2 cm × 2.5 cm) equilibrated with 0.1 M-formic acid. The column was washed with formic acid until the 280 nm trace returned to the baseline and the column then was washed with water. All the radioactivity was bound to the column under these conditions. The radioactive peptides were finally eluted with 0.3 M-NH₃ and the sample concentrated by rotary flash evaporation to a small volume for t.l.c.

Iodination of proteins

The method was adapted from that of Hunter & Greenwood (1962). First 1 ml of a 1 mg/ml solution of protein was dialysed for 16 h against 0.2 M-sodium phosphate buffer, pH 7.5, containing 0.5 mM-EDTA and 0.1% SDS. Then 1 mCi of ¹²⁵I (carrier-free; The Radiochemical Centre) was added, followed by

10 μ l of chloramine-r solution (5mg/ml). After a few seconds the reaction was stopped by the addition of 100 μ l of sodium metabisulphite solution (2.4mg/ml) and 100 μ l of KI solution (1mg/ml). Low-molecular-weight radioactive material was removed by overnight dialysis against 0.5mM-EDTA, and finally by overnight dialysis against 10mM-Tris/HCl buffer, pH8.0.

Trypsin treatment

The samples in the 10mM-Tris/HCl buffer, pH8.0, were treated with trypsin for 24h at 37°C by the addition of 100 μ l of a 1mg/ml solution of pig trypsin (Miles Laboratories, Stoke Poges, Bucks., U.K.) at zero time and after 6h. Pig trypsin has the advantage of having a very low chymotrypsin activity.

Peptide 'mapping'

The tryptic digest was fractionated on silica thin-layer plates (Polygram SIL N-HR; Camlab, Cambridge, U.K.). The mixture was fractionated in the first dimension in ethanol/conc. NH_3 (sp.gr. 0.88) (7:3, v/v), the plate dried and electrophoresed in the second dimension for 4h at 15V/cm in a pyridine/acetic acid/water (1:10:189, by vol.) solvent at pH3.5.

The radioactive peptides were finally located by using Agfa-Gevaert Curix RP 1 film.

As relatively large samples of carboxymethylated peptides were applied to the t.l.c. plates, great care was taken to remove non-protein material extracted from the homogenized polyacrylamide slabs, which interfered with the chromatography (see above). The interference was caused by very small particles of gel which are not removed by centrifugation, but which can be separated from peptides by gel-exclusion chromatography, and a soluble component, which, unlike peptides, is not bound to SE-cellulose at acid pH. The charge properties of this soluble material (it is bound to DEAE-cellulose at alkaline pH) suggest it to be acrylic acid. As smaller samples of iodinated peptides need to be applied to the t.l.c. plates, the non-protein contaminants did not interfere with the separation.

Results

Amino acid analysis

Fig. 1 shows that the amino acid analysis of bovine spectrin gives values very similar to those published by other laboratories for other species. Amino acid

Fig. 1. Comparison of the amino acid analyses of spectrin from several mammalian species ●, Ox (the present paper); ▲, human; △, sheep; ○, horse; ■, guinea pig (Tillack *et al.*, 1970).

Table 1. *Amino acid analyses of spectrin and peptides P1 and P2*

Cysteine and methionine were determined as cysteic acid and the sulphone in spectrin. Insufficient material was available for these analyses for peptides P1 and P2, and the values (*) for the two acids are those obtained directly from the ninhydrin analysis of the column eluate from the total acid digest.

	Spectrin (EDTA extract)	Peptide P1	Peptide P2
Lys	6.1	7.8	6.5
His	2.3	2.4	2.9
Arg	4.7	7.7	6.9
	13.1	17.9	16.3
Asp	11.9	10.2	10.3
Glu	19.9	18.9	20.3
	31.8	29.1	30.6
Thr	3.9	4.6	4.5
Ser	5.4	5.8	4.9
Pro	2.8	4.2	2.9
Gly	5.6	4.8	6.5
Ala	8.2	8.1	8.8
Cys	1.2	0.6*	0.3*
	27.1	28.1	27.9
Met	2.4	1.7*	1.9*
Val	4.7	4.0	4.5
Ile	3.7	3.5	3.4
Leu	10.8	11.4	11.4
Tyr	1.9	1.8	1.1
Phe	3.7	2.5	2.9
	27.2	24.9	25.2

composition is of only limited value for the characterization of an unknown protein, and if used uncritically can lead to erroneous conclusions. Ketterer *et al.* (1973) identified characteristic features in the composition of any given protein by comparing its distribution of amino acids with the distributions in 200 known proteins. On this basis spectrin is distinguished by its unusually high content of glutamic acid and, to a lesser degree, leucine. The relative proportions of these two acids is significantly greater than their values in analyses of the total protein complement of the membrane.

The ratio of amino N to aspartate+glutamate content indicates that no more than 60% of the side-chain carboxyl groups are amidated.

Membrane proteins are, in general, characterized by their relatively high contents of hydrophobic amino acids (Capaldi & Vanderkooi, 1972). This is a manifestation of the extent of the interaction of the polypeptide chains with the hydrophobic domain of the membrane lipids. As spectrin is an extrinsic

protein, it would not therefore be expected to have a high content of hydrophobic amino acids. In spectrin the 27% hydrophobic amino acids may be compared with the 33% of the major intrinsic protein of the erythrocyte membrane, P3 (Ho & Guidotti, 1975; Jenkins & Tanner, 1977).

When the amino acid compositions of the major peptides of spectrin, P1 and P2, are compared, they are found to be remarkably similar to each other and to the unfractionated parent extract (Table 1). All three have the diagnostically high glutamate and leucine contents. These data confirm the previously published incomplete analyses of the two polypeptides from human erythrocytes (Brandon, 1975).

Peptide 'mapping'

Tryptic digests of spectrin peptides P1 and P2 have been analysed after labelling the proteins with either [¹⁴C]carboxymethyl side chains or [¹²⁵I] catalysed by chloramine-T (Figs. 2, 3 and 4). Line drawings of the photographs are provided to illustrate some weak spots that are clear on the original radioautographs, but some of which do not reproduce well in photographs. Spots common to peptides P1 and P2 are indicated, though common spots can differ in intensity between the two polypeptides. The identification of common spots is based on our interpretation of these 'maps' and similar 'maps' made from other preparations of peptides P1 and P2. In most cases the identity can be claimed with confidence, but in such complicated 'maps' as the iodinated ones the possibility of coincidence must always be taken into consideration.

The 'maps' of carboxymethylated peptides contain 20–25 peptides, which is consistent with the polypeptides being single chains of very approx. 200000 daltons (assuming an average mol. wt. of 120 for amino acids, a residue present at 1 mol% would generate around 20 peptides from a 240000-dalton polypeptide). Secondly, the 'maps' of peptides P1 and P2 share several common peptides, but they are not identical.

The latter results is obtained from the 'maps' of the iodinated proteins, i.e. peptide P1 is very similar to peptide P2. Again the 'maps' are not identical, and they summate to that of the whole extract. The same result was obtained whether purified peptides P1 and P2 were iodinated or if the EDTA extract was iodinated and the radioactive peptides P1 and P2 were subsequently isolated. (Samples were routinely reduced before iodination. A control in which the reduction was immediately followed by carboxymethylation with non-radioactive iodoacetic acid and then iodinated in the presence of chloramine-T was identical with those that had not been carboxymethylated.)

Clearly both labels demonstrate the similarity, but

Fig. 2. Comparison of the [^{14}C]carboxymethylated peptides in tryptic digests of purified peptides P1 and P2

The 'maps' were prepared as described in the Experimental section. Weak spots in the radioautographs are represented by thin lines in the line drawings. In the drawing of the 'map' of peptide P1 those peptides also present in peptide P2 are indicated with cross-hatching, and vice versa. (a) Radioautograph of peptide P1; (b) radioautograph of peptide P2; (c) line drawing of (a); (d) line drawing of (b).

not identity, of the two chains. Many peptides are common to both polypeptides, but some are peculiar to peptide P1 and others to peptide P2. The presence of the same peptides in 'maps' of both proteins cannot be attributed to cross-contamination, for analytical gels of the separated peptides P1 and P2 (Fig. 3) show no trace of cross-contamination. Coomassie Blue staining would detect a contamination of at least 10%, probably less, and the similarities between the 'maps' could not be caused by such low degrees of contamination. The presence of peptides peculiar to either of the two fractions is additional evidence against cross-contamination.

We cannot detect any proteolytic activity in the spectrin preparation by using standard protein substrates such as Azocoll or Azoalbumin, but a slow breakdown of peptides P1 and P2 over a period of weeks may be observed by SDS/polyacrylamide-gel electrophoresis. The degradation is affected by bivalent ions. If an extract is dialysed against 5mM-Tris/HCl or 2.5mM-sodium phosphate at pH7.5 for 48 h at 18°C it breaks down, but if 0.5mM-EDTA is added to the diffusate the spectrin is stable for

several days. The degradation is increased by several bivalent ions (Cu^{2+} , Zn^{2+} , Fe^{2+}), but is not affected by Ca^{2+} or Mg^{2+} . Furthermore, if Cu^{2+} ions are added to the extract, degradation can take place even after the addition of 5% SDS, heating at 100°C for 5 min and adding urea to 8M. One might at first attribute these results to bacterial contamination, but none can be detected, and the incubations are always performed in the presence of kanamycin sulphate [0.5mg/ml; Sigma (London) Chemical Co., Kingston upon Thames KT2 7BH, U.K.]. We have not found any wholly satisfactory explanation for these findings, but they have led us to monitor all preparations for degradation before use. As shown in Fig. 5, the fractions used in the experiments show no trace of degradation.

Discussion

The amino acid analyses and the peptide 'maps' constructed from carboxymethylated and from iodinated proteins all show the close similarity of peptides P1 and P2. This similarity has previously

Fig. 3. 'Map' of the tryptic peptide from ox spectrin labelled with ^{125}I by using chloramine-T

The 'map' was prepared as described in the Experimental section. Weak spots in the radioautograph are represented by thin lines in the line diagram. (a) Radioautograph; (b) line drawing of (a).

been inferred from immunological data and *N*-terminal analyses of these same fractions, but both these approaches also led to the conclusion that peptides P1 and P2 were heterogeneous. The relative simplicity of the present 'maps', particularly those of carboxymethylated proteins, confirm the more widely held belief that peptides P1 and P2 are single polypeptides of high molecular weight. How may this conflict be resolved?

Multiplicity of *N*-termini in the EDTA extract has been reported by three laboratories (Knufermann *et al.*, 1973; Fuller *et al.*, 1974; Dunn *et al.*, 1975), and the latter two groups found that the *N*-termini of peptides P1 and P2 were identical. The fractions used for the *N*-terminal analyses of Dunn *et al.* (1975) were

prepared by the same methods used in the present work, i.e. they had been reduced, boiled with SDS and shown to co-migrate with the peptides P1 and P2 of freshly prepared 'ghosts'. We (Dunn *et al.*, 1975) therefore concluded that the most probable explanation for the *N*-terminal data was that the 'polypeptides' were in fact stable aggregates of several smaller subunits. However, we, and others, e.g. Pinder *et al.* (1976), have not been able subsequently to dissociate peptides P1 and P2 except under conditions where proteolysis, although unlikely, could not be rigorously excluded, and evidence for covalent crosslinks has not been forthcoming (Bailey *et al.*, 1976). Alternatively, the multiplicity of *N*-termini could be due to the presence of several chains of identical size co-migrating with peptides P1 and P2. Both these explanations are now improbable, as they would predict more complicated peptide 'maps' than those obtained. As SDS has occasionally been found not to dissociate protein complexes (Maddy, 1976), the possibility of small amounts of adventitiously bound small peptides cannot be totally dismissed. It remains possible that the multiplicity of *N*-termini might be due to exopeptidases cleaving a few terminal amino acids from the polypeptides without significantly lowering the molecular weights of the remaining chains. The same *N*-terminal pattern in the two polypeptides could arise from their having similar or identical *N*-terminal sequences, a conclusion consistent with our present data.

Immunological comparison of the two polypeptides has produced conflicting results. On the one hand Bjerrum *et al.* (1975) concluded that the two were similar. The fused-rocket-immunoelectrophoresis technique indicated that both peptides P1 and P2 contain at least two antigens, and crossed immunoelectrophoresis revealed that peptides P1, P2 and P2.1 share the same antigens, but there was no cross-reaction with any other membrane polypeptides. On the other hand Sheetz *et al.* (1976) report an antibody for peptide P1, which does not cross-react with peptide P2.

It has been claimed that spectrin contains actin and myosin. The actin has been identified with peptide P5 (Tilney & Detmers, 1975) and is not relevant to the present discussion. It is present in insufficient quantity to affect the amino acid composition of the whole EDTA extract significantly or make a marked contribution to the peptide 'map' of the extract. We cannot detect any methylhistidine in the extract. However, the possibility of myosin being a significant component of spectrin is highly relevant, especially as we confirm (M. J. Dunn & A. H. Maddy, unpublished-work) Brandon's (1975) observation that rabbit myosin heavy chain co-migrates with peptide P2. The early reports of a complete actomyosin system in the erythrocyte

Fig. 4. Comparison of the tryptic digests of [^{125}I]iodinated peptides P1 and P2

The large radioactive area seen in the second dimension is an iodinated product of the polyacrylamide gel used for the preparation of peptides P1 and P2. In the line drawing of the 'map' of peptide P1 those peptides also present in peptide P2 are indicated with cross-hatching, and vice versa. (a) Radioautograph of peptide P1; (b) radioautograph of peptide P2; (c) line drawing of (a); (d) line drawing of (b).

(Ohnishi, 1962; Jirgl, 1971) are doubtful, as the erythrocyte 'ghosts' were almost certainly contaminated with platelets, which are rich in thrombos-thenin. The amino acid composition of myosin heavy chain and spectrin are similar (Guidotti, 1972), but in our system the tryptic-peptide 'map' of iodinated myosin is clearly distinguished from the 'maps' of the EDTA extract, peptides P1 and P2. It may also be pointed out that the amino acid-composition evidence applies to both peptides P1 and P2, yet only the latter has the same mobility as myosin. Immunological analysis does not indicate the presence of myosin. Sheetz *et al.* (1976) report that anti-spectrin does not cross-react with myosin, although a weak

reaction was detectable between antibody against uterine myosin and spectrin. These workers found little or no actin-stimulated Mg^{2+} -dependent adenosine triphosphatase in the erythrocyte membrane. They concluded that spectrin is not identical with myosin, although it may share some common antigenic determinants. It may be noted that spectrin is extracted at low, not high, ionic strength and bears no morphological resemblance to myosin (Harris & Maddy, 1974; Tilney & Detmers, 1975).

Fukui *et al.* (1971) observed common peptides shared by many different proteins extracted from *Micrococcus lysodeikticus* membranes and concluded that these represented parts of the proteins

Fig. 5. Analytical gels of fractions P1 and P2 isolated by preparative polyacrylamide-gel electrophoresis as described in the text

(a) Unfractionated EDTA extract; (b) purified peptide P1; (c) a fraction from the preparative gel, intermediate in position between peptides P1 and P2, containing both polypeptides; (d) purified peptide P2.

which were buried in the hydrophobic lipid domain of the membrane *in vivo* and were of similar structure, because they all fulfilled the common function of anchoring the proteins into the membrane. Such an explanation is not likely for the spectrin polypeptides, which are extrinsic proteins. The biological significance of the presence of two very similar polypeptides in the spectrin complex is obscure. Little is known of the arrangement of peptides P1 and P2 within the membrane, but the EDTA extract is known to contain particles of the order of 480000 mol.wt. (Ralston, 1975; Schechter *et al.*, 1976), a fact which, together with the results of work with cross-linking reagents (Hulla & Gratzner, 1972) suggests that in free solution, and possibly in the intact membrane, peptides P1 and P2 exist as a dimer which is the functional unit. One of the next stages in the investigation will be the separation of peptides P1 and P2 under conditions that do not lead to irreversible denaturation, so that their separate roles may be assessed. In view of their chemical similarity as well as their large size, this is likely to be a formidable task.

We are indebted to the Medical Research Council for financial support and to Mr. J. Meredith for technical assistance.

References

- Bailey, A. J., Robins, S. P. & Tanner, M. J. A. (1976) *Biochim. Biophys. Acta* **434**, 51–57
- Bjerrum, O. J., Bhakdi, S., Bøg-Hansen, T. C. & Knufermann, H. (1975) *Biochim. Biophys. Acta* **406**, 489–504
- Brandon, D. L. (1975) *FEBS Lett.* **58**, 349–352
- Capaldi, R. A. & Vanderkooi, G. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 930–932
- Dunn, M. J., McBay, W. & Maddy, A. H. (1975) *Biochim. Biophys. Acta* **386**, 107–119
- Elgsaeter, A., Shotton, D. M. & Branton, D. (1976) *Biochim. Biophys. Acta* **426**, 101–122
- Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617
- Fuller, G. M., Boughter, J. M. & Morazzani, M. (1974) *Biochemistry* **13**, 3036–3041
- Fukui, Y., Nachbar, M. S. & Salton, M. R. J. (1971) *Biochim. Biophys. Acta* **241**, 30–37
- Guidotti, G. (1972) *Annu. Rev. Biochem.* **41**, 731–752
- Guthrow, C. E., Allen, J. E. & Rasmussen, H. (1972) *J. Biol. Chem.* **247**, 8145–8153
- Harris, J. R. & Maddy, A. H. (1974) *J. Ultrastruct. Res.* **48**, 190–200
- Harris, J. I. & Perham, R. N. (1965) *J. Mol. Biol.* **13**, 876–884
- Hirs, C. H. W. (1967) *Methods Enzymol.* **11**, 197–198
- Ho, M. K. & Guidotti, G. (1975) *J. Biol. Chem.* **250**, 675–683
- Holy, H. W. (1966) in *Techniques in Amino Acid Analysis* (Schmidt, D. I., ed.), pp. 127–133, Technicon, Chertsey
- Hulla, F. W. & Gratzner, W. B. (1972) *FEBS Lett.* **25**, 275–278
- Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496
- Jenkins, R. E. & Tanner, M. J. A. (1977) *Biochem. J.* **161**, 139–147
- Jirgl, V. (1971) *Folia Biol. (Prague)* **17**, 392–399
- Johnson, P., Harris, C. J. & Perry, S. V. (1967) *Biochem. J.* **105**, 361–370
- Ketterer, B., Hicks, R. M., Christodoulides, L. & Beale, D. (1973) *Biochim. Biophys. Acta* **311**, 180–186
- Knufermann, H., Bhakdi, S., Schmidt-Ullrich, R. & Wallach, D. F. H. (1973) *Biochim. Biophys. Acta* **330**, 356–361
- Maddy, A. H. (1966) *Biochim. Biophys. Acta* **117**, 193–200
- Maddy, A. H. (1976) *J. Theor. Biol.* **62**, 315–326
- Maddy, A. H. & Dunn, M. J. (1974) *Protides Biol. Fluids Proc. Colloq.* **21**, 21–26
- Marchesi, V. T. & Steers, E. (1968) *Science* **159**, 203–204
- Ohnishi, T. (1962) *J. Biochem. (Tokyo)* **52**, 307–316
- Pinder, J. C., Tidmarsh, S. & Gratzner, W. B. (1976) *Arch. Biochem. Biophys.* **172**, 654–660
- Ralston, G. B. (1975) *Aust. J. Biol. Sci.* **28**, 259–266
- Schechter, N. M., Sharp, M., Reynolds, J. A. & Tanford, C. (1976) *Biochemistry* **15**, 1897–1904

- Shapiro, D. L. & Marchesi, V. T. (1977) *J. Biol. Chem.* **252**, 508–517
- Sheetz, M., Painter, R. G. & Singer, S. J. (1976) in *Cell Motility* (Goldman, R. D., Pollard, T. D. & Rosenbaum, J. L., eds.), pp. 651–664, Cold Spring Harbor Publications, Cold Spring Harbor
- Steck, T. L. (1974) *J. Cell Biol.* **62**, 1–19
- Tillack, T. W., Marchesi, S. L., Marchesi, V. T. & Steers, E. (1970) *Biochim. Biophys. Acta* **200**, 125–131
- Tilney, L. G. & Detmers, P. (1975) *J. Cell Biol.* **66**, 508–520
- Tristram, G. R. (1966) in *Techniques in Amino Acid Analysis* (Schmidt, D. I., ed.), pp. 61–72, Technicon, Chertsey