Release of C-terminal tyrosine from tubulin and microtubules at steady state

Carlos A. ARCE and Héctor S. BARRA

Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), UNC-CONICET, Facultad de Ciencias Químicas, Casilla de Correo 61, 5016 Córdoba, Argentina

(Received 2 October 1984/Accepted 15 October 1984)

Microtubule protein preparations purified by cycles of assembly–disassembly contain the enzyme tubulinyltyrosine carboxypeptidase (TTCPase). Using these preparations, containing tubulinyl[^{14}C]tyrosine, we studied the release of [^{14}C]tyrosine from assembled and non-assembled tubulin under steady-state conditions. It was found that both states of aggregation were detyrosinated at similar rates by the action of the endogenous TTCPase. However, practically no release of [^{14}C]tyrosine from the non-assembled tubulin pool was found when microtubules were previously eliminated from the incubation mixture. These results indicated that non-assembled tubulin requires to interact with microtubules to be detyrosinated. This interaction seems to occur through the incorporation of dimers into microtubules, since when the capability of tubulin to incorporate into microtubules was diminished by binding of colchicine a concomitant decrease in the rate of release of tyrosine was observed. When detyrosination was accelerated by increasing the concentration of TTCPase relative to the microtubule protein concentration, microtubules were found to be detyrosinated faster than was non-assembled tubulin. Using exogenous TTCPase in an incubation system in which the formation of microtubules was not allowed, tubulinyl[^{14}C]tyrosine and tubulinyl[^{14}C]tyrosine–colchicine complex were shown to have similar capabilities to act as substrates for this enzyme. Free colchicine was shown not to affect the activity of TTCPase.

The post-translational modification of tubulin which involves the addition or release of a tyrosine residue at the C-terminus of the α -chain has been previously described (Barra *et al.*, 1974; Arce *et al.*, 1975; Hallak *et al.*, 1977; Raybin & Flavin, 1977; Argaraña *et al.*, 1980). The product of the incorporation reaction, tubulinyltyrosine, has apparently the same capacity as non-tyrosinated tubulin to assemble into microtubules *in vitro* (Arce *et al.*, 1978; Kumar & Flavin, 1982). However, it was reported that in brain tissue from new-born rats the proportion of tyrosinated tubulin in the non-assembled fraction is higher than that in the microtubule fraction (Rodríguez & Borisy, 1979). It is possible that the ratio tyrosinated/non-tyrosinated tubulin in both states of aggregation depends not only on the relative capabilities of

both species of tubulin to assemble into microtubules but also on the relative capabilities of both states of aggregation to act as substrates of the tyrosinating enzyme, tubulin:tyrosine ligase (TTLase) and the detyrosinating enzyme, tubulinyltyrosine carboxypeptidase (TTCPase) (Arce *et al.*, 1978). In a previous work we showed that TTLase incorporates tyrosine into tubulin dimers but not into microtubules (Arce *et al.*, 1978). This was confirmed by Deanin *et al.* (1980). On the other hand, several authors (Hallak *et al.*, 1977; Thompson, 1977; Deanin *et al.*, 1980; Kumar & Flavin, 1981) have reported that microtubules are the preferred substrate for the TTCPase. Recently, we have reported that TTCPase associates with microtubules *in vitro* in such a way that microtubule protein purified by up to five cycles of assembly–disassembly contains TTCPase activity (Arce & Barra, 1983).

The present work deals with the action of endogenous TTCPase on both assembled and non-assembled tubulin under steady-state conditions.

Abbreviations used: TTCPase, tubulinyltyrosine carboxypeptidase; TTLase, tubulin:tyrosine ligase; MTP, microtubule protein.

Materials and methods

Materials

L-[U- 14 C]Tyrosine (sp. radioactivity 450 μ Ci/ μ mol) was obtained from New England Nuclear. Sephadex G-25 (28–80 μ m), DEAE-Sephadex A-50, Pipes, Mes and EGTA were purchased from Sigma. Colchicine was purchased from Merck.

Brain extract preparation

Brains from 30–60-day-old rats were homogenized in 1 vol. of Pipes buffer (0.1M-Pipes adjusted with KOH to pH6.7, containing 1mM-EGTA). The homogenate was centrifuged at 100000g for 1h at 2–4°C and the supernatant solution was immediately used.

Preparation of [14 C]tyrosinated microtubule protein by assembly–disassembly

For the incorporation of [14 C]tyrosine into tubulin the incubation mixture contained per ml: 0.9ml of the brain extract, 150 μ mol of KCl, 2.5 μ mol of ATP, 8 μ mol of $MgCl_2$ and 2.2–4.4nmol (1–2 μ Ci) of [14 C]tyrosine. After incubation for 20min at 37°C the mixture was cooled and passed through a column of Sephadex G-25 equilibrated with Pipes buffer to eliminate unbound [14 C]tyrosine. The excluded material was mixed with 1 vol. of non-labelled brain extract and, after addition of glycerol [40% (v/v) final concn.], GTP (1mM) and $MgCl_2$ (0.5mM), tubulin was allowed to assemble by incubating the mixture at 37°C for 30min. After centrifugation at 100000g for 30min at 27°C the pellet was resuspended in Mes buffer (0.1M-Mes adjusted with KOH to pH6.7, containing 1mM-EGTA) to give a protein concentration of about 3mg/ml. After 30min at 0°C the suspension was centrifuged at 100000g for 10min at 2–4°C. The supernatant solution (microtubule protein purified by one cycle) was designated MTP($\times$ 1). Subsequent assembly–disassembly cycles were carried out in Mes buffer in the presence of 1mM-GTP and 0.5mM- $MgCl_2$. The incubation time for assembly was 15min and the warm centrifugation to separate the assembled from the non-assembled fraction was at 100000g for 10min. The purified microtubule protein preparations MTP($\times$ 1), MTP($\times$ 2) and so forth were immediately used: $\times$ 1, $\times$ 2, etc. indicate the number of the assembly–disassembly cycles. The post-assembly supernatant fraction obtained after warm centrifugations were designated S_1 , S_2 , etc.

When unlabelled microtubular protein was required, it was obtained as described for the labelled microtubular protein except that the incorporation of [14 C]tyrosine was omitted.

Measurement of the radioactivity bound to protein

To an aliquot of the sample to be analysed, 2ml of 5% trichloroacetic acid was added; the mixture was heated at 90°C for 5min and the radioactivity bound to protein was measured in the hot-trichloroacetic acid-insoluble material (Barra *et al.*, 1973).

Determination of the release of [14 C]tyrosine catalysed by the endogenous TTCPase

Unless otherwise specified the [14 C]tyrosinated MTP preparation was incubated at 37°C in the presence of 1mM-GTP and 0.5mM- $MgCl_2$. After the stated incubation period the radioactivity bound to protein was measured as described above. The radioactivity release was calculated as the difference between a zero-time control and the experimental tube. All determinations were done in duplicate.

Preparation of pure tubulinyll [14 C]tyrosine and tubulinyll [14 C]tyrosine–colchicine complex

A [14 C]tyrosinated MTP($\times$ 1) preparation was obtained as described above except that before the incorporation of [14 C]tyrosine the brain extract was passed through a column of Sephadex G-25 and that 11nmol (5 μ Ci) was the amount of [14 C]tyrosine added/ml of incubation system. Then, the [14 C]tyrosinated MTP($\times$ 1) preparation was chromatographed on a column of phosphocellulose as described by Sloboda & Rosenbaum (1982) except that Mes buffer was used instead of Pipes. The [14 C]tyrosinated tubulin collected in the excluded volume was more than 95% pure as judged by polyacrylamide-gel electrophoresis performed according to Weber & Osborn (1969). No TTCPase activity was found in this preparation. To obtain the tubulinyll [14 C]tyrosine–colchicine complex, a fraction of the purified [14 C]tyrosinated tubulin preparation was incubated in the presence of 0.2mM-colchicine for 2h at 37°C in the darkness and immediately passed through a column of Sephadex G-25 equilibrated with Mes buffer to eliminate unbound colchicine. Both preparations were immediately used.

Purification of TTCPase

A 400-fold purified preparation of TTCPase was obtained from rat brain as described by Modesti *et al.* (1984).

Polyacrylamide-gel electrophoresis

The continuous sodium dodecyl sulphate gel system described by Weber & Osborn (1969) was used. The gels (7.5% acrylamide, 0.097% bisacrylamide) were 0.8cm $\times$ 10cm in size. After running they were stained with Coomassie Brilliant Blue and destained by diffusion.

Turbidimetric measurements

Assembly of tubulin was monitored turbidimetrically at 350nm in a Shimadzu double-beam spectrophotometer (model UV-150-02). Temperature was maintained at 37°C with a circulating water bath. Assembly was initiated by transferring the microtubular protein preparation to the pre-warmed cuvettes in the carousel.

Electron microscopy

Aliquots (5 μ l) of microtubule solutions were allowed to settle for 20s on carbon-coated Formvar grids. The grids were washed successively with 3 drops of water and 3 drops of 1% uranyl acetate, and the excess uranyl acetate was drawn off with filter paper. Grids were examined after they had been air-dried.

Protein determination

Proteins were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Results and discussion

Detyrosination of tubulinyl[14 C]tyrosine isolated from the assembled and non-assembled fractions

It is known that in nervous tissue there are several isotypes of tubulin (Gozes & Littauer, 1978; Marotta *et al.*, 1978; Dahl & Weibel, 1979). To determine whether the tubulinyl[14 C]tyrosine that remains in the non-assembled fractions is similar to that obtained from disassembled microtubules with respect to its capability to release [14 C]tyrosine, the following experiment was carried out. [14 C]Tyrosinated MTP($\times 2$) and S_2 preparations were mixed with unlabelled S_2 and MTP($\times 2$) preparations, respectively, to obtain two systems of similar final composition and then incubated at 37°C to determine the release of [14 C]-tyrosine by the action of the endogenous TTCPase. It should be noted that under these conditions the formation of microtubules was permissible. The release of [14 C]tyrosine was similar for both mixtures (Fig. 1) indicating that, in this respect, there are no differences between the molecules of tubulinyl[14 C]tyrosine obtained from the assembled fraction and those from the non-assembled fraction.

Release of [14 C]tyrosine from assembled and non-assembled tubulinyl[14 C]tyrosine under steady-state conditions

The release of [14 C]tyrosine was carried out in a system in which both assembled and non-assembled tubulinyl[14 C]tyrosine were present. Assembly was performed by incubating a [14 C]-tyrosinated MTP($\times 2$) preparation in Mes buffer in

Fig. 1. Release of [14 C]tyrosine from tubulinyl[14 C]tyrosine obtained from the assembled and non-assembled fractions

A sample (2ml) of a [14 C]tyrosinated MTP($\times 1$) preparation (3mg/ml) was processed to obtain the labelled MTP($\times 2$) (assembled tubulin) and S_2 (non-assembled tubulin) fractions. Identical amounts of unlabelled MTP($\times 2$) and S_2 fractions were also prepared. The total amounts of labelled and unlabelled MTP and S fractions were mixed as follows: Labelled MTP($\times 2$) plus unlabelled S_2 (O) and labelled S_2 plus unlabelled MTP($\times 2$) (●). The mixtures were incubated at 37°C and at the stated times the [14 C]tyrosine released was measured.

the presence of 1 mM-GTP and 0.5 mM-MgCl₂. The formation of microtubules during the incubation was monitored by turbidimetry and checked by electron microscopy. In our experimental conditions an apparent steady state was reached after 5min of incubation. To measure the release of [14 C]tyrosine in these conditions no addition of TTCPase was necessary since the microtubule protein preparation contains enough enzyme activity (Arce & Barra, 1983). After different periods of incubation, aliquots (0.3ml) were removed and centrifuged to separate the assembled and non-assembled fractions. Aliquots removed at 5, 20 and 60min of incubation were shown to contain the same proportion (about 75%) of assembled protein. The specific radioactivity (c.p.m. bound to protein/mg of protein) was measured in the assembled and non-assembled fractions (Fig. 2). To compare the rate of decay of the specific radioactivity of assembled tubulin with that of non-assembled tubulin, the values were expressed as percentages (Fig. 2, inset). Tubulin in either state of aggregation was detyrosinated at a similar rate. The difference in the specific radioactivity between assembled and non-assembled fractions observed in Fig. 2 is due, in part, to a dissimilar distribution of non-labelled proteins. Polyacrylamide-gel electrophoresis revealed that some microtubule-associated proteins, such as the high M_r proteins are present only in the assembled fraction (results

Fig. 2. Detyrosination of assembled and non-assembled tubulin at steady state

A [^{14}C]tyrosinated MTP($\times 2$) preparation (3 mg/ml) was incubated in assembly conditions (1 mM-GTP, 0.5 mM-MgCl₂, 37°C) and at the stated times, 0.3 ml aliquots were removed and centrifuged at 100 000g for 10 min at 27°C. The specific radioactivity (c.p.m. bound to protein/mg of protein) was measured in the assembled (○) and non-assembled (●) fractions. Experimental values are the mean of three independent experiments. Inset: values were expressed as percentages with reference to the specific radioactivity at 5 min of incubation.

not shown). In addition, during the warm centrifugation, [^{14}C]tyrosine is released from the pelleted microtubules faster than from the non-assembled tubulin dimers (see below), contributing in this way to the difference in specific radioactivity between both fractions.

Detyrosination of non-assembled tubulin after depletion of microtubules

When microtubules at steady state were eliminated from the incubation system, the non-assembled tubulin fraction was detyrosinated at a very low rate. This result was obtained from the following experiment. A [^{14}C]tyrosinated MTP($\times 2$) preparation was incubated at 37°C in the assembly conditions described above and after 15 min the microtubules were spun down. Imme-

Table 1. Release of [^{14}C]tyrosine from non-assembled tubulin in a system depleted of microtubules

A [^{14}C]tyrosinated MTP($\times 2$) preparation was incubated in assembly conditions for 15 min and microtubules were eliminated by centrifugation. The microtubule-depleted supernatant fraction was further incubated for 60 min at 37°C to measure the release of [^{14}C]tyrosine. For comparison, the release of [^{14}C]tyrosine from a MTP($\times 2$) preparation was also measured. An identical procedure was carried out with [^{14}C]tyrosinated MTP($\times 3$) and MTP($\times 4$) preparations. Experimental values are the mean for the determinations in three different experiments.

Preparation	Release of [^{14}C]tyrosine (%)
MTP($\times 2$)	41
Microtubule-depleted MTP($\times 2$)	4
MTP($\times 3$)	39
Microtubule-depleted MTP($\times 3$)	3
MTP($\times 4$)	34
Microtubule-depleted MTP($\times 4$)	4

diately, the supernatant solution containing the non-assembled tubulin fraction was incubated at 37°C for 60 min and the release of [^{14}C]tyrosine was measured. A similar procedure was carried out with MTP($\times 3$) and MTP($\times 4$) preparations. In Table 1 the release of [^{14}C]tyrosine from the microtubule-depleted fraction can be compared with that corresponding to the complete system (non-assembled tubulin plus microtubules at steady state). In all cases, the release from the non-assembled fraction was about 10-fold lower than that from the complete system. The rate of release of [^{14}C]tyrosine from microtubules in the pelleted form was similar to that from the complete system (results not shown). These results indicate that under steady-state conditions, the non-assembled tubulin pool requires to interact with microtubules to be detyrosinated.

Detyrosination of the non-assembled tubulin pool under steady-state conditions

As mentioned above, non-assembled tubulin requires to interact with microtubules to be detyrosinated. We considered alternative mechanisms by which this interaction can occur. One is by simple collisions between non-assembled dimers and the wall of microtubules. The other one is by assembly of the tubulin dimers into the microtubule ends. In connection with this question the following experiment was carried out. An unlabelled MTP($\times 2$) preparation was allowed to assemble and, after reaching the steady state, a trace of labelled substrate (tubulinyl[^{14}C]tyrosine or tubulinyl[^{14}C]tyrosine-colchicine complex) was added and the incubation continued. Aliquots

were removed after different periods of time and centrifuged to separate the assembled and non-assembled fractions. Protein and radioactivity bound to protein were determined in each fraction. As can be seen in Table 2 the proportion of protein in the assembled state measured after addition of the tubuliny[^{14}C]tyrosine–colchicine complex was practically the same as that measured when tubuliny[^{14}C]tyrosine was the labelled substrate added. However, the distribution of the radioactivity between the assembled and non-assembled

fractions were dissimilar (Fig. 3). The proportion of radioactivity found in the microtubular fraction was lower when tubuliny[^{14}C]tyrosine–colchicine complex was the labelled substrate, indicating that this complex has a diminished ability to assemble into microtubules in comparison with tubuliny[^{14}C]tyrosine. Under these conditions the release of [^{14}C]tyrosine from tubuliny[^{14}C]tyrosine–colchicine was lower than that from tubuliny[^{14}C]tyrosine (Fig. 4). The possibility that this result was due to a lower ability of tubuliny[^{14}C]tyrosine–colchicine complex to act as substrate of TTCFase was ruled out, since both tubuliny[^{14}C]tyrosine and tubuliny[^{14}C]tyrosine–colchicine complex

Fig. 3. Distribution of tubuliny[^{14}C]tyrosine and tubuliny[^{14}C]tyrosine–colchicine complex between the assembled and non-assembled tubulin fractions

An unlabelled MTP($\times 2$) preparation (3 mg/ml) was incubated in assembly conditions and after 15 min an aliquot (5% of final volume) of pure tubuliny[^{14}C]tyrosine (0.95 mg/ml) (○) or tubuliny[^{14}C]tyrosine–colchicine complex (0.87 mg/ml) (●) was added and the incubation continued. Aliquots (0.3 ml) were removed at the stated times and centrifuged to separate the assembled and non-assembled fractions. Protein and radioactivity bound to protein were determined. Radioactivity in the pellet was expressed as the percentage with respect to total (radioactivity in the pellet plus that in the supernatant).

Fig. 4. Release of [^{14}C]tyrosine after addition of tubuliny[^{14}C]tyrosine (○) or tubuliny[^{14}C]tyrosine–colchicine complex (●) to a preparation of microtubules at steady state

Protocol and experimental conditions were identical to those described in Fig. 3 except that after addition of the labelled substrates, aliquots (50 μl) were removed at the stated times to measure the [^{14}C]tyrosine released as described in the Materials and methods section.

Table 2. Distribution of protein between the assembled and non-assembled fractions
Protocol and experimental conditions were those described in Fig. 3.

Addition	Incubation time (min)	Protein (mg) in:	
		Assembled fraction	Non-assembled fraction
Tubuliny[^{14}C]tyrosine	5	0.61	0.29
	25	0.66	0.27
	45	0.65	0.22
Tubuliny[^{14}C]tyrosine–colchicine	5	0.58	0.31
	25	0.62	0.27
	45	0.61	0.26

were shown to be detyrosinated at the same rate when tested with exogenous enzyme in an incubation system where no microtubules are formed (Fig. 5a). In addition, the presence of free colchicine in the incubation system did not affect the activity of the enzyme (Fig. 5b). Therefore, the decrease in the rate of detyrosination observed when the capability of the radioactive substrate to assemble into microtubules was diminished supports the idea that the detyrosination of the non-assembled tubulin pool occurs via assembly into microtubules. If the release of [14 C]tyrosine from the non-assembled tubulin pool occurred via simple collisions with the microtubule walls, the rate of release of [14 C]tyrosine should be the same

Fig. 5. Effect of colchicine on the release of [14 C]tyrosine from pure tubulin/[14 C]tyrosine

(a) Effect of colchicine bound to tubulin. Tubulinyl-[14 C]tyrosine (○) (20 μ g, 12000 c.p.m.) or tubulinyl-[14 C]tyrosine-colchicine complex (●) (18 μ g, 11000 c.p.m.) and TTCpase (10 μ g) were incubated at 37°C in a system containing 100 mM-Mes buffer, pH 6.7, 1 mM-GTP and 2 mM-MgCl₂. Final volume, 0.1 ml. (b) Effect of free colchicine. Tubulinyl-[14 C]tyrosine (20 μ g, 12000 c.p.m.) and TTCpase (10 μ g) were incubated at 37°C in a system containing 100 mM-Mes buffer, pH 6.7, 1 mM-GTP and 2 mM-MgCl₂ in the absence (○) or in the presence (●) of 0.1 mM-colchicine. Final volume, 0.1 ml. At the stated times the radioactivity bound to protein was measured and the [14 C]tyrosine released was expressed as the percentage with respect to a zero-time control.

whatever the distribution of the labelled substrate between the assembled and non-assembled fractions.

Since the intrinsic capability of the tubulin molecule to be detyrosinated is not altered by the binding of colchicine, the use of this drug allowed us to estimate the relative capabilities of assembled and non-assembled tubulin to act as substrates of TTCpase. The release of [14 C]tyrosine from a [14 C]tyrosinated MTP($\times 2$) preparation was measured in the absence or presence of 0.1 mM-colchicine. From results in Fig. 6 it can be inferred that the release from microtubules is at least 2.5-fold faster than that from non-assembled tubulin.

Effect of increasing TTCpase concentrations on the release of tyrosine from tubulin and microtubules at steady state

Considering our results, the detyrosination of assembled and non-assembled tubulin at steady state by the action of the endogenous TTCpase would be as follows. TTCpase is mostly associated with microtubules and catalyses the release of tyrosine from the assembled tubulin. The non-assembled tubulin pool is only detyrosinated after assembly into microtubules. The finding of similar rates of detyrosination for the assembled and non-assembled tubulin pools (Fig. 2) implies that the exchange of tubulin dimers between tubulin and microtubules should be very rapid in comparison with the rate of detyrosination. If the rate of

Fig. 6. Effect of colchicine on the release of [14 C]tyrosine from a [14 C]tyrosinated microtubule protein preparation. A [14 C]tyrosinated MTP($\times 2$) preparation (3 mg/ml) was incubated in assembly conditions in the absence (○) or in the presence (●) of 0.1 mM-colchicine. At the stated times the [14 C]tyrosine released was determined as described in the Materials and methods section.

Table 3. Effect of increasing TTC Pase concentrations on the release of tyrosine from tubulin and microtubules at steady state

A [^{14}C]tyrosinated MTP($\times 2$) preparation (2.5 mg/ml) was incubated in assembly conditions in the presence of purified TTC Pase at the indicated concentrations. After 5 and 20 min of incubation, 0.3 ml aliquots were removed and centrifuged at 100000g for 10 min at 27°C. The specific radioactivity (c.p.m. bound to protein/mg of protein) was measured in the assembled and non-assembled fractions. The decay of the specific radioactivity from 5 to 20 min of incubation indicated the release of [^{14}C]tyrosine that occurred during the 15 min interval.

Added TTC Pase (mg/ml)	[^{14}C]Tyrosine released (%)	
	Tubulin	Microtubules
0	15	14
0.06	15	18
0.18	18	38
0.36	21	49

detyrosination were higher than that of exchange, microtubules should be more detyrosinated than non-assembled tubulin. This hypothesis was confirmed by measuring the release of [^{14}C]tyrosine at increasing concentrations of TTC Pase relative to the MTP concentration. Results from a typical experiment are shown in Table 3.

These results taken together indicate that the tyrosination state of tubulin and microtubules depends not only on the relative capabilities of both states of aggregation to act as substrates of TTC Pase but also on the rate of exchange of tubulin dimers. In addition, since TTC Pase appears to act almost exclusively on microtubules and TTLase on non-assembled tubulin (Arce *et al.*, 1978; Deanin *et al.*, 1980; Kumar & Flavin, 1981), it becomes evident that the turnover of the C-terminal tyrosine is dependent upon assembly-disassembly of tubulin. This is compatible with a previous report by Thompson *et al.* (1979) who found in living cells in culture that intact microtubules are required for rapid turnover of the C-terminal tyrosine.

We are grateful to Dr. R. Caputto, Dr. J. A. Curtino and Ing. M. A. Aón for critical review of the manuscript. This work was supported in part by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas, the Subsecretaría de Ciencia y Tecnología and the Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba, Argentina.

References

- Arce, C. A. & Barra, H. S. (1983) *FEBS Lett.* **157**, 75–78
- Arce, C. A., Rodríguez, J. A., Barra, H. S. & Caputto, R. (1975) *Eur. J. Biochem.* **59**, 145–149
- Arce, C. A., Hallak, M. E., Rodríguez, J. A., Barra, H. S. & Caputto, R. (1978) *J. Neurochem.* **31**, 205–210
- Argaña, C. E., Barra, H. S. & Caputto, R. (1980) *J. Neurochem.* **34**, 114–118
- Barra, H. S., Rodríguez, J. A., Arce, C. A. & Caputto, R. (1973) *J. Neurochem.* **20**, 97–108
- Barra, H. S., Arce, C. A., Rodríguez, J. A. & Caputto, R. (1974) *Biochem. Biophys. Res. Commun.* **60**, 1384–1390
- Dahl, J. L. & Weibel, V. J. (1979) *Biochem. Biophys. Res. Commun.* **86**, 822–828
- Deanin, G. G., Preston, S. F., Hanson, R. K. & Gordon, M. W. (1980) *Eur. J. Biochem.* **109**, 207–216
- Gozes, I. & Littauer, U. Z. (1978) *Nature (London)* **276**, 411–413
- Hallak, M. E., Rodríguez, J. A., Barra, H. S. & Caputto, R. (1977) *FEBS Lett.* **73**, 147–150
- Kumar, N. & Flavin, M. (1981) *J. Biol. Chem.* **256**, 7678–7686
- Kumar, N. & Flavin, M. (1982) *Eur. J. Biochem.* **128**, 215–222
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Marotta, C. A., Harris, J. L. & Gilbert, J. M. (1978) *J. Neurochem.* **30**, 1431–1440
- Modesti, N. M., Argaña, C. E., Barra, H. S. & Caputto, R. (1984) *J. Neurosci. Res.*, in the press
- Raybin, D. & Flavin, M. (1977) *Biochemistry* **16**, 2189–2194
- Rodríguez, J. A. & Borisy, G. G. (1979) *Biochem. Biophys. Res. Commun.* **89**, 893–899
- Sloboda, R. D. & Rosenbaum, J. L. (1982) *Methods Enzymol.* **85**, 409–416
- Thompson, W. C. (1977) *FEBS Lett.* **80**, 9–13
- Thompson, W. C., Deanin, G. G. & Gordon, M. W. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1318–1322
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412