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Differential Protein Insertion Into Developing Photosynthetic Membrane Regions of Rhodopseudomonas sphaeroides

Gordon S. Inamine, Patricia A. Reilly, and Robert A. Niederman

Department of Biochemistry, Bureau of Biological Research, Rutgers University, Piscataway, New Jersey 08854

Previous studies have suggested that much of the B800-850 light-harvesting bacteriochlorophyll *a*-protein complex is inserted directly into the intracytoplasmic photosynthetic membrane of Rhodopseudomonas sphaeroides. In contrast, the B875 light-harvesting and reaction center complexes are assembled preferentially at peripheral sites of photosynthetic membrane growth initiation. The basis for this apparent site-specific polypeptide insertion was examined during the inhibition of RNA and protein syntheses. The pulse labeling of polypeptides at the membrane growth initiation sites was significantly less sensitive to inhibition by rifampicin, chloramphenicol, or kasugamycin than in the intracytoplasmic or outer membranes. This suggests increased stability for the translation machinery at these membrane invagination sites. Similar differential effects in polypeptide insertion were observed during inhibition of bacteriochlorophyll synthesis through deprival of δ-aminolevulinate to R sphaeroides mutant H-5, which requires this porphyrin precursor. The pulse-labeling patterns observed during the inhibition of both RNA and pigment syntheses were consistent with the uncoupling of polypeptide insertion into the membrane invagination sites from their growth and maturation into intracytoplasmic membranes.

Key words: Rhodopseudomonas sphaeroides, light-harvesting complexes, reaction centers, membrane assembly

In reponse to lowered oxygen tension, the facultative photoheterotrophic bacterium Rhodopseudomonas sphaeroides produces an extensive intracytoplasmic photosynthetic membrane (ICM) system thought to be continuous with the cytoplasmic membrane (CM) [1–3]. Upon disruption of ICM-containing cells in a French press, three distinct membrane fractions can be isolated by direct rate-zone sedimentation in sucrose density gradients [4, 5]. These consist of: (1) an upper pigmented band with ~5–14% of the total bacteriochlorophyll *a* (Bchl); (2) ICM-derived chromatophore vesicles which contain the remaining Bchl, and (3) an unpigmented cell wall-enriched

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fraction. Results obtained from radiolabeling [4–6], cellular fractionation [5], photochemical [7], and fluorescence yield [8] studies have suggested that pigmented membranes isolated in the upper band are derived from photosynthetic membrane growth-initiations sites in which reaction centers and their associated B875 light-harvesting complexes are assembled preferentially. In contrast, much of the accessory B800–850 light-harvesting antenna is inserted directly into the ICM [5,6]. Furthermore, pulse-chase studies [4,5] suggested that these transient membrane growth-initiation sites ultimately mature into ICM with the isolation characteristics of chromatophores.

In the present study, this apparent site-specific insertion of pigment-protein complexes into developing photosynthetic membranes has been examined further during inhibition of transcription, translation, and Bchl synthesis. The results suggest distinct physiological responses in the various membrane domains and differences in assembly mechanisms for the pigment-protein complexes at these sites.

MATERIALS AND METHODS

Growth of Organisms and Isolation of Membrane Fractions

R sphaeroides NCIB 8253 was grown photoheterotrophically at 30°C with a light intensity of 1,800 lux as described previously [9]. For photosynthetic growth of the R sphaeroides H-5 mutant (kindly provided by J. Takemoto), 1.0 mM δ-aminolevulinate (δ-ALA) was included in the medium. This mutant, derived from the NCIB 8253 strain, requires δ-ALA for porphyrin biosynthesis [10]. Cell-free extracts were prepared in a French-pressure cell and the upper pigmented, chromatophore, and cell-wall-enriched fractions were isolated by rate-zone sedimentation as in [4] on 5–35% (wt/wt) sucrose gradients prepared over a cushion of 60% (wt/wt) sucrose. The upper pigmented band was purified further as in [11] using a 5–55% (wt/wt) sucrose density gradient centrifuged for 100 min at 200,000g_{avg} in a Beckman SW40Ti rotor.

Pulse-Labeling Procedure

Exponentially growing cells were treated with antibiotics as specified in the text and culture aliquots were pulse labeled with L-[^{35}S]methionine (1.27 Ci/mol; Amersham Corp.) for 5 min under the above conditions of phototrophic growth. Labeling was terminated as in [4]. For studies on Bchl synthesis, phototrophically grown H-5 cells were pelleted, washed in 0.9% NaCl, resuspended in fresh growth medium lacking δ-ALA, and subjected to the above growth conditions. Aliquots removed at the specified intervals were labeled as described above.

Electrophoretic Separation of Membrane Proteins

The isolated membrane fractions were solubilized and subjected to sodium dodecyl sulfate (SDS)/polyacrylamide gel electrophoresis as in [9]. The separating gel slabs were formed with gradients of 10–20% or 7.5–15% acrylamide. Membrane protein concentrations were estimated as in [12]. The protein concentrations of the membrane fractions were equalized in each gel lane. Autoradiography of the stained and dried gel slabs was performed as in [11]. Radioactivity of the polypeptide bands was estimated from the developed autoradiograms with an Ortec model 4310 scanning densitometer.

RESULTS

The effects of specific antibiotics on the pulse labeling of membrane polypeptides were examined in an effort to determine whether differences in the protein synthetic machinery exist at distinct regions of the CM-ICM continuum. We first investigated the potential of mRNA to direct the synthesis of protein destined for insertion into these different membrane regions in the presence of the transcription inhibitor rifampicin. Phototrophically growing cells were pulse labeled with L-[^{35}S]methionine at various intervals during a 30-min rifampicin treatment, membrane fractions were isolated and their polypeptide components resolved by SDS/polyacrylamide gel electrophoresis (Fig. 1) [9,11]. In agreement with previous results [4,5], the rate of pulse labeling in the upper pigmented band was about twofold greater than in the other membrane fractions. During the early stages of rifampicin treatment, the association of newly synthesized protein with membranes in the upper pigmented band was more resistant than in the other fractions; eg, after 6 min, the initial labeling rate increased slightly in the upper fraction while declining by about 20% in chromatophores. Thereafter, labeling declined at approximately the same rate in these two membrane fractions, as indicated by the similar half-lives for the decay in their overall protein insertion (Table I). The increased stability of the protein synthetic machinery at the membranes of the upper pigmented band was seen more dramatically upon examination of the polypeptide components of the pigment-protein complexes, especially those of the reaction center, in which an approximately 1.5-fold increase in the rate of labeling was seen over the first 6 min. As observed with the overall membrane fractions, once this resistance to rifampicin was overcome, the pulse labeling of the Bchl-associated polypeptides in the upper pigmented and chromatophore fractions decayed at approximately the same rate. A somewhat shorter half-life was observed in the pulse labeling of the overall cell-wall fraction; that of the major outer membrane protein component [16–18] was 9 min, which approaches the 7 min

TABLE I. Half-lives for Rifampicin-Induced Decay in Membrane Protein Insertion

Fraction	Polypeptide	Half-life[a] (min)
Upper pigmented	-	21.9
	LH-2	23.1
Chromatophore	-	21.5
	LH-1	16.8
	LH-2	18.5
Cell wall	-	14.7
	42 kd[b]	9.1

[a]Calculated from linear portion of decline in pulse-labeling rates. These values are only indirect estimates of mRNA half-lives, since they may also reflect translation events and the association of the translation products with the membrane.

[b]This is the major outer membrane protein component [16–18], which migrated with a 42,000 M_r [16] in our gel system.

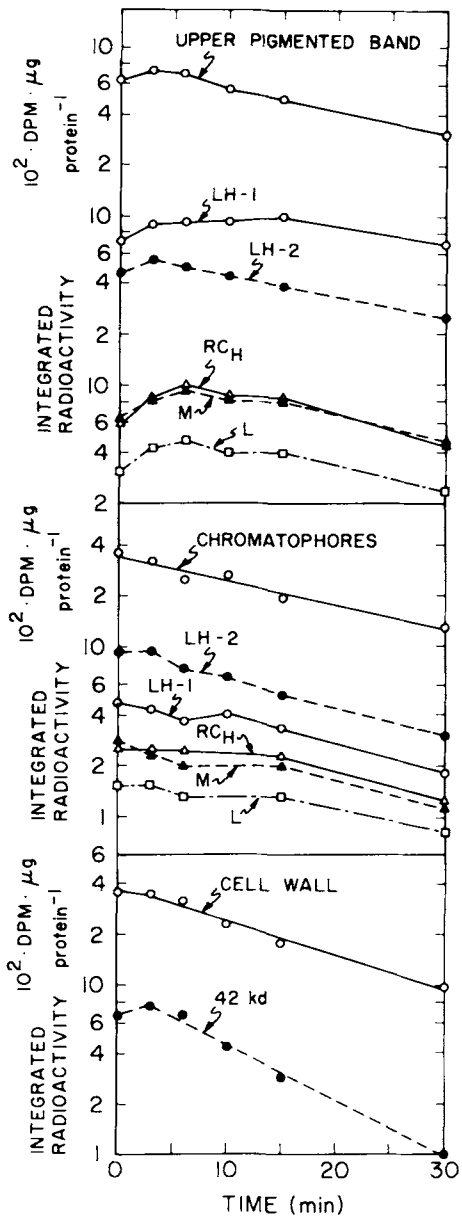

Fig. 1. Protein-labeling patterns during inhibition of RNA synthesis. Rifampicin was added to phototrophically growing R sphaeroides NCIB 8253 at 0 min to a final concentration of 200 μg/ml, and the cells were pulse labeled at the designated intervals with L-[^{35}S]methionine (0.25 μCi/ml). Membrane fractions were isolated, their radioactivity determined in a Beckman LS-3150T liquid scintillation counter as in [4], and the results expressed as DPM·μg protein^{-1}. The radioactivity of their designated polypeptide bands after SDS/polyacrylamide gel electrophoresis was quantitated from peak heights in densitometric scans of the resulting autoradiograms [11]. The results are expressed as integrated radioactivity in arbitrary units. Each gel lane received 80 μg of membrane protein. RC_H, RC_M, RC_L, denote reaction center polypeptide subunits, which migrate with M_r = 28,000, 24,000, and 21,000, respectively [13, 14]; LH-1, LH-2, are partially resolved polypeptide subunits of the B875 and B800-850 light-harvesting Bchl complexes, respectively, which migrate with respective M_r = 12,000 and 10,000 [15]. Most of the pulse labeling was confined to these polypeptides. An additional polypeptide component migrating with a M_r near 11,000 that does not appear to be associated with Bchl was observed under conditions in which improved resolution of low M_r polypeptides was achieved. The high level of pulse labeling of this component in the upper pigmented band also resisted the early stages of rifampicin treatment.

reported recently for the soluble protein fraction of the related organism R capsulata [19].

More dramatic differences in the pulse-labeling rates between the upper pigmented and the other membrane fractions were observed with kasugamycin, an inhibitor of polypeptide chain initiation [20], and chloramphenicol, which inhibits the peptidyl transferase-catalyzed polypeptide chain elongation step [20] (Fig. 2). With the highest kasugamycin concentration, protein incorporation was decreased by only about 10% in the upper pigmented band, whereas decreases of approximately 34% and 30% were observed in the chromatophore and cell wall fractions, respectively. Likewise, the pulse labeling of the upper pigmented band was significantly less sensitive to inhibition by chloramphenicol; at the highest antibiotic concentration, incorporation was decreased by about 64% and 65%, respectively, in chromatophore and cell wall fractions, and by approximately 42% in the upper pigmented band. Similar differences in the effects of these translation inhibitors were observed for the polypeptide components of pigment-protein complexes after SDS/polyacrylamide gel electrophoresis of the upper pigmented and chromatophore fractions (not shown, [21]). Overall, these results further support the possibility that site-specific insertion of protein into the separable regions of the CM-ICM continuum may be related to differences in the mechanisms of membrane protein synthesis and assembly.

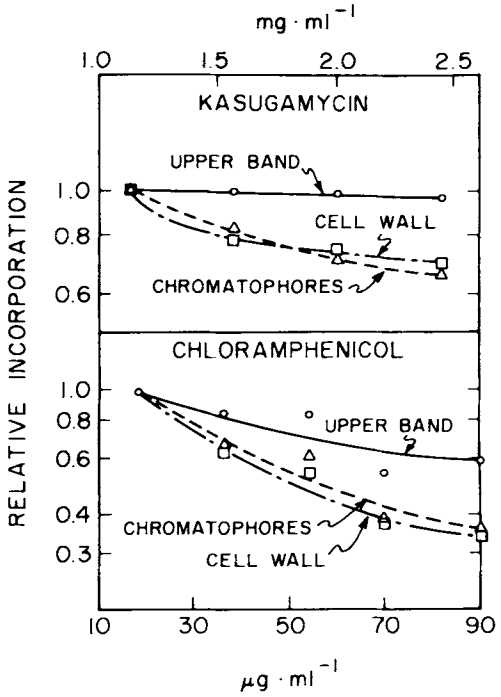

Fig. 2. Differential effects of translation inhibitors in membrane fractions. Individual aliquots were removed from phototrophically growing R sphaeroides NCIB 8253, treated with antibiotic for 6-7 min, and pulse labeled with L-[^{35}S]methionine (0.25 μCi/ml). Specific radioactivities for the isolated membrane fractions were determined as described in Figure 1 and are expressed as incorporation rates relative to values of 1.0 at antibiotic concentrations where no significant inhibitory effects were observed.

The role of continued Bchl synthesis in the site-specific assembly of pigment-protein complexes in the different CM-ICM regions was examined further in the R sphaeroides H-5 mutant, which requires δ-ALA for porphyrin biosynthesis [10]. A previous labeling study with δ-[^{3}H]ALA during a shift-down in light intensity suggested that Bchl is synthesized and assembled preferentially into pigment-protein complexes at photosynthetic membrane growth initiation sites [6]. To determine the absolute dependence of the insertion of these apoprotein components into the different membrane domains upon continued Bchl synthesis, cells were pulse labeled with L-[^{35}S]methionine at several periods following δ-ALA deprival (Fig. 3). The association of several apparent reaction center and light-harvesting polypeptides with the upper pigmented band resists δ-ALA removal for up to 4 hr. In contrast, their appearance in chromatophores was much more sensitive to the decreased δ-ALA concentration and only the apparent B800-850-associated LH-2 polypeptide was significantly resistant after 3 hr. It is possible that small amounts of residual δ-ALA are sufficient for

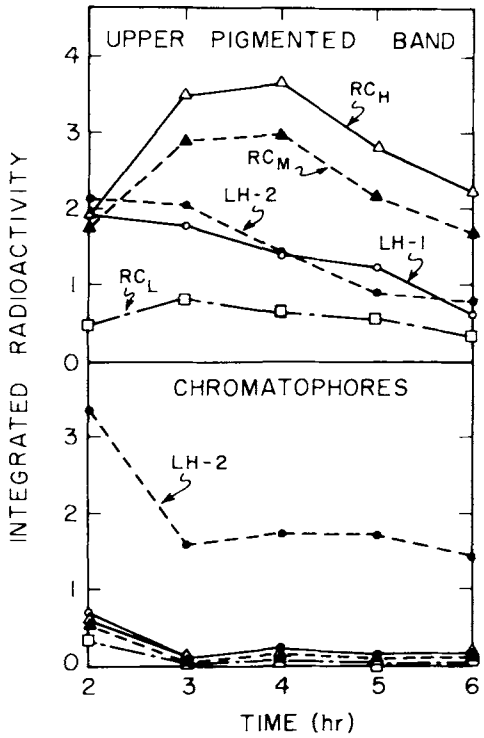

Fig. 3. Pulse labeling of membrane proteins during δ-ALA deprival of R sphaeroides H-5. Phototrophically grown cells were pulse labeled with L-[^{35}S]methionine (0.4 μCi/ml) at the indicated intervals after removal of δ-ALA. Membrane fractions were isolated and subjected to SDS-polyacrylamide gel electrophoresis. The designations of the polypeptide bands and the methods for quantitation of their radioactivity are presented in Figure 1. Each lane of the gel slabs received 70 μg of membrane protein. After 3 hr in the absence of δ-ALA, the Bchl level in the culture, determined as in [9], decreased from 0.80 to 0.59 μg/ml.

some Bchl synthesis that is responsible for the continued site-specific appearance of these polypeptides. This will be discussed in more detail below. During restoration of δ-ALA to deprived cells, essentially the same pattern of differential protein insertion was observed in the upper pigmented and chromatophore fractions (not shown).

DISCUSSION

There is now considerable evidence to support the hypothesis [4,5] that the upper pigmented fraction is derived in part from sites of peripheral CM invagination at which growth of the ICM is initiated. This includes (1) pulse-chase studies with several amino acids in both R sphaeroides [4,5] and the related organism Rhodospirillum rubrum [11], which suggested that reaction center and B875 polypeptides are inserted preferentially into these membrane regions which mature to ICM with isolation characteristics of chromatophores; (2) evidence from other labeling studies, which indicated that in pigmented membranes of the upper band, Bchl is synthesized preferentially and the completed pigment-protein complexes reside transiently [6]; (3) surface labeling [22] and membrane fractionation studies [5], which indicated a peripheral localization for these membranes as well as the presence of relatively unpigmented peripheral CM [16] within this fraction; and (4) spectroscopic characterization of this fraction, which indicated that the size and composition of the photosynthetic units [7], their stage of assembly [6] and fluorescence yield properties [8] were those expected of photosynthetic membranes early in their development.

Freeze-etch studies [23] have suggested that in addition to the initiation of ICM growth at new sites on the CM, the ICM is also expanded by the elongation of existing invaginations. Indeed, studies in synchronous R sphaeroides cells suggest that the ICM is a major site for the insertion of new protein throughout the cell cycle [24–26]. Although under these circumstances, the pigment-protein complexes are accumulated at the same rates and in constant proportions [24,25], during a shift from high to low intensity, the rate of pulse labeling of B800-850 polypeptides is accelerated relative to that of the B875 and reaction center protein [27] and the B800-850 components are inserted preferentially into the ICM [6]. The possible basis for such differential labeling and site-specific assembly of pigment-protein complexes in the growing ICM and at photosynthetic membrane growth initiation sites has been addressed in the present study.

The studies with rifampicin, kasugamycin, and chloramphenicol were consistent with a role for the translation machinery in the site-specific incorporation of polypeptides into the different membrane domains. The pulse labeling of protein at apparent membrane growth initiation sites was more resistant to inhibition by kasugamycin and chloramphenicol than in the intracytoplasmic or outer membranes; this suggests greater stability for the initiation and elongation of polypeptide chains destined for these sites. Pulse labeling at these membrane growth sites was also more resistant to rifampicin; inhibition by this antibiotic was delayed for as much as 10 min. Paradoxically, pulse-labeling rates of apparent reaction center and B875 polypeptides at such sites were actually increased by as much as 1.5-fold during this period. It is possible that during the early stages of rifampicin treatment, mRNA associated with the translation machinery at these membrane growth points was protected from degradation. Under such circumstances, either mRNA translation or the membrane associa-

tion of the translation products was rate-limiting for the observed pulse labeling. Thereafter, as the total mRNA pool became depleted during the continued inhibition of transcription, these reduced mRNA levels may have become rate-limiting. This may account for the similarity in decay of pulse labeling observed in the upper pigmented and chromatophore fractions once resistance to rifampicin was overcome.

The increases in pulse labeling rates observed during the first 6–10 min after rifampicin addition may reflect the transient existence of pigment-proteins at the membrane growth initiation sites and the movement of polypeptides into and out of these sites. Accordingly, both the effects of the initial polypeptide insertion and the maturation of these growth initiation sites to larger invaginations with the isolation characteristics of chromatophores may be reflected in the pulse labeling. It has been suggested [5, 6] that such membrane growth may occur largely through direct addition of B800-850 to these preformed regions. Since pulse labeling of the B800-850 LH-2 polypeptide component and of the overall ICM was more sensitive to rifampicin, it is possible that growth of the initial invagination sites was also inhibited by the antibiotic. This could therefore account for the observed increases in the accumulation of pulse-labeled polypeptides in the upper pigmented fraction.

The assembly of the pigment-protein complexes in the different domains of the CM-ICM continuum was also examined in the δ-ALA-requiring H-5 mutant. Marked differences in the pulse labeling of these polypeptides were also observed between the upper pigmented and chromatophore fractions during δ-ALA deprival; this was accompanied by an overall decrease in cellular Bchl levels. Similar to the rifampicin effects, a nearly twofold increase in pulse labeling was observed for apparent reaction center polypeptides during the first 3–4 hr of deprival, while that in the ICM was markedly decreased. Since it has been shown that completed Bchl molecules are necessary for the appearance of these polypeptides in the membrane [28], it is possible that any remaining δ-ALA available for Bchl synthesis or any free pigment might be responsible for the observed effects. Normally, only a small pigment-protein pool exists at the membrane growth initiation sites relative to that in the ICM and any continued pulse labeling would be observed more readily in the former. Moreover, these conditions could restrict ICM growth and might ultimately prevent movement of protein via membrane expansion away from the initial invagination sites, which could again account for the observed accumulation of pulse labeled protein.

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REFERENCES

1. Niederman RA, Gibson KD: In Clayton RK, Sistrom WR (eds): "The Photosynthetic Bacteria." New York: Plenum, 1978, pp 79–118.
2. Kaplan S: In Clayton RK, Sistrom WR (eds): "The Photosynthetic Bacteria." New York: Plenum, 1978, pp 809–835.
3. Drews G, Oelze J: Adv Microbiol Physiol 22:1, 1981.
4. Niederman RA, Mallon DE, Parks LC: Biochim Biophys Acta 555:210, 1979.

5. Niederman RA, Hunter CN, Inamine GS, Mallon DE: In Akoyunoglou G (ed): "Photosynthesis," Vol V, Chloroplast Development. Philadelphia: Balaban International Science Services, 1981, pp 663–674.
6. Hunter CN, Pennoyer JD, Niederman RA: In Akoyunoglou G, Evangelopoulos AE, Georgatsos J, Palaiologos G, Trakatellis A, Tsiganos CP (eds): "Cell Function and Differentiation, Part B, Biogenesis of Energy Transducing Membranes and Membrane and Protein Energetics." New York: Alan R. Liss, Inc., 1982, pp 257–265.
7. Hunter CN, Holmes NG, Jones OTG, Niederman RA: Biochim Biophys Acta 548:253, 1979.
8. Hunter CN, van Grondelle R, Holmes NG, Jones OTG, Niederman RA: Photochem Photobiol 38:313, 1979.
9. Broglie RM, Niederman RA: J Bacteriol 138:788, 1979.
10. Lascelles J, Altshuler T: J Bacteriol 98:721, 1969.
11. Inamine GS, Niederman RA: J Bacteriol 150:1145, 1982.
12. Markwell MAK, Haas SM, Bieber LL, Tolbert NE: Anal Biochem 87:206, 1978.
13. Clayton RK, Haselkorn R: J Mol Biol 68:97, 1972.
14. Okamura MY, Steiner LA, Feher G: Biochemistry 13:1394, 1974.
15. Broglie RM, Hunter CN, Delepelaire P, Niederman RA, Chua NH, Clayton RK: Proc Natl Acad Sci USA 77:87, 1980.
16. Parks LC, Niederman RA: Biochim Biophys Acta 511:70, 1978.
17. Deal CD, Kaplan S: J Biol Chem 258:6524, 1983.
18. Deal CD, Kaplan S: J Biol Chem 258:6530, 1983.
19. Dierstein R, Drews G: In Akoyunoglou G, Evangelopoulos AE, Georgatsos J, Palaiologos G, Trakatellis A, Tsiganos CP (eds): "Cell Function and Differentiation, Part B, Biogenesis of Energy Transducing Membranes and Membrane and Protein Energetics." New York: Alan R. Liss, Inc., 1982, pp 247–256.
20. Pestka S: Annu Rev Microbiol 25:487, 1971.
21. Inamine GS: Ph.D. Thesis, Rutgers University, 1983.
22. Inamine GS, Van Houten J, Niederman RA: J Bacteriol (submitted).
23. Golecki JR, Oelze J: J Gen Microbiol 88:253, 1975.
24. Fraley RT, Lueking DR, Kaplan S: J Biol Chem 253:458, 1978.
25. Wraight CA, Lueking DR, Kaplan S: J Biol Chem 253:465, 1978.
26. Fraley RT, Lueking DR, Kaplan S: J Biol Chem 254:1980, 1979.
27. Chory J, Kaplan S: J Bacteriol 153:465, 1983.
28. Takemoto J, Lascelles J: Proc Natl Acad Sci USA 70:799, 1973.