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Inhibition of Adenosine Triphosphatase Activity of the Plasma Membrane Fraction of Oat Roots by Diethylstilbestrol[1]

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ABSTRACT

Diethylstilbestrol (DES) inhibited noncompetitively the ATPase in the plasma membrane fraction from *Avena sativa* L. cv. Goodfield roots when assayed in the presence of $MgSO_4$ or $MgSO_4$ plus KCl. In the presence of $MgSO_4$, 7.1×10^{-5} molar DES inhibited the enzyme 50%; whereas in the presence of $MgSO_4$ and KCl, 1.3×10^{-4} molar DES was required for the same inhibition. Dixon plots indicated that in the presence of $MgSO_4$, one molecule of DES bound to one molecule of ATPase; however, in the presence of $MgSO_4$ and KCl, two or more molecules bound to one ATPase molecule. These results suggested that KCl causes a conformational change in the enzyme which exposes additional binding sites for DES, but that these sites are not as inhibitory as the first binding site.

In addition to KCl, other factors also affected the DES inhibition of the ATPase. Plasma membrane vesicles warmed to 38 C were inhibited more than vesicles kept on ice prior to assay. DES inhibited the Triton X-100-treated ATPase less than the ATPase which was not detergent-treated. Finally, studies with DES analogs showed that the hydroxyl groups of DES were essential for inhibition and that steric configurations of the molecule were important.

DES inhibition of the ATPase suggests that DES inhibits K^+ absorption in oat roots by inhibiting the ATPase. Inhibition of K^+ absorption was greater than inhibition of the ATPase, and thus DES may also inhibit other aspects of metabolism that are involved with ion absorption.

The rapid inhibition of ion absorption in oat roots by DES[3] (3) suggested that DES inhibited absorption at the plasma membrane, or it moved rapidly into the cytoplasm and inhibited some other metabolic activity. Because ATPase enzymes are believed to be involved with ion transport across the plasma membrane (14), we have investigated the effects of DES on the ATPase activity of the plasma membrane fraction of oat roots. Previous work showed that 10^{-4} M DES inhibited the ATPase of oat roots about 50% (1); transport ATPases of animal cells were also inhibited by DES (13, 20, 23).

In this paper we report experiments designed to characterize the DES inhibition of the ATPase activity of the plasma membrane fraction from oat roots. We show that DES decreased the Mg^{2+}-, $(Mg^{2+}+K^+)$-, and ΔK^+-ATPase activities of the plasma membrane fraction. The kinetic data of DES inhibition of the various ATPase activities were different. Also, the Triton X-100-treated ATPase was less sensitive than the untreated enzyme to DES.

MATERIALS AND METHODS

Oat (*Avena sativa* L. cv. Goodfield) seeds were germinated and grown over aerated 1.0 mM $CaSO_4$ as previously described (15). Plasma membrane vesicles were isolated from oat roots by differential and discontinuous gradient centrifugation (15). Modifications of that procedure included: *(a)* inclusion of 1 mM DTT in all solutions; *(b)* suspension of microsomal pellets in 15% instead of 18% sucrose; and *(c)* centrifugation on gradients consisting of layers of 45, 34, 30, and 25% (w/w) sucrose. In some experiments plasma membrane vesicles (34/45% interface) were treated with 0.05% Triton X-100, 12 mM $Tris_L$-MES_L (pH 6.5) for 15 min at 26 C in an attempt to solubilize the ATPase. The Triton-treated vesicles were centrifuged at 96,000*g* for 1 hr, and the supernatant was assayed for ATPase activity.

ATPase activities were determined by measuring Pi hydrolyzed from ATP. Pi was determined by the Dulley (8) modification of the Fiske and SubbaRow (11) assay. Reaction medium consisted of ATP (Tris salt [pH 6.5]), $MgSO_4$, and Tris-MES (pH 6.5); KCl and inhibitors were added in certain experiments. $Tris_L$-MES_L concentration was 33 mM unless stated otherwise. Specific concentrations of other constituents are given in figure legends and tables. Assay tubes were incubated 15 min at 38 C; this temperature gave nearly maximum activity of the Mg^{2+}-ATPase (26). ΔK^+-ATPase was calculated as follows: $(Mg^{2+}+K^+)$-ATPase minus Mg^{2+}-ATPase = ΔK^+-ATPase.

Protein content of the plasma membrane fractions was estimated by the procedure of Lowry *et al.* (19) after precipitation of the protein with 10% trichloroacetic acid at 8 C for 20 hr. Crystalline BSA was the protein standard. Proteins in the presence of 0.05% Triton X-100 were precipitated in 0.1% SDS, 10% trichloroacetic acid at 8 C for 20 hr (modified procedure of Dulley and Grieve [9]) prior to the Lowry assay.

All phenolic compounds were purchased from Sigma Chemical Co., and were dissolved in absolute ethanol. The final concentration of ETOH was 1% in all assays including controls. DES analogs precipitated at concentration above 10^{-4} M. This posed no problem for the Pi determination because the SDS added in the Pi assay solubilized the hydrophobic precipitates and prevented interference with the assay.

Results are the mean of at least two experiments with each treatment in triplicate. The standard error of the mean was less than 3% of the mean for each experiment.

RESULTS

At 38 C, activity of the plasma membrane Mg^{2+}-ATPase in the presence of 10^{-4} M DES was linear with time (Fig. 1); activity in the presence of 1% ETOH (control) was linear through only the

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[3] Abbreviations: DES: diethylstilbestrol; ETOH: ethanol; K_i: inhibition constant; Mg^{2+}-ATPase: ATPase assayed in the presence of $MgSO_4$; $(Mg^{2+}+K^+)$-ATPase: ATPase assayed in the presence of $MgSO_4$ and KCl; ΔK^+-ATPase: KCl-stimulated component of ATPase, calculated as $(Mg^{2+}+K^+)$-ATPase minus Mg^{2+}-ATPase; $Tris_L$-MES_L: buffer made by mixing solutions of equal concentrations of Tris and MES to give desired pH.

first 10 min of incubation. Twice as much protein (about 30 μg/assay) as usual was employed in this experiment in order to have sufficient activity at the shorter times. In subsequent experiments, we used 10 to 15 μg protein/assay and a 15-min assay. These conditions produced linearity for both DES and ETOH treatments.

At concentrations above 10^{-5} M, DES dramatically reduced the Mg^{2+}-ATPase activity of the plasma membrane fraction (Fig. 2). At 7.1×10^{-5} M DES, ATPase activity was 50% of the control, and at 10^{-3} M DES, the ATPase was practically inactive. A small but consistent 3 to 4% stimulation was observed at 10^{-6} M DES.

DES also decreased (Mg^{2+}+K^+)-ATPase and ΔK^+-ATPase activities of the plasma membrane fraction (Fig. 3). At 10^{-6} M and 10^{-3} M DES, ΔK^+-ATPase activity was decreased slightly more than Mg^{2+}-ATPase activity. At DES concentrations between 10^{-5} and 10^{-4} M, ΔK^+-ATPase was not quite as sensitive as Mg^{2+}-ATPase. DES at 1.3×10^{-4} inhibited ΔK^+-ATPase 50%.

Mg^{2+}-, (Mg^{2+}+K^+)-, and ΔK^+-ATPase activities were measured at several DES concentrations between 10^{-5} and 10^{-4} M. A Dixon plot of Mg^{2+}-ATPase activities showed an intersection of straight lines on the abscissa (Fig. 4A); this result indicated noncompetitive inhibition of the enzyme by DES (6). The K_i was 1.1×10^{-4} M. However, Dixon plots of (Mg^{2+}+K^+)-ATPase (Fig. 4B) and ΔK^+-ATPase (Fig. 4C) showed lines that were parallel as they approached the ordinate but curved upward as the DES concentra-

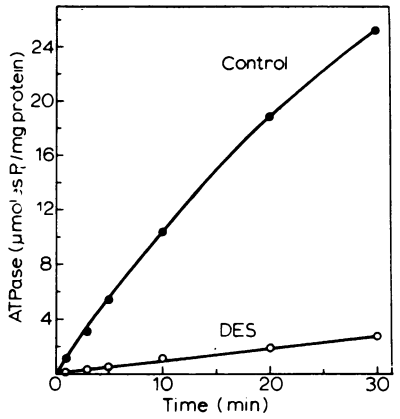

FIG. 1. Time course of (Mg^{2+}+K^+)-ATPase activity at 38 C in the presence of DES or ETOH. Assay medium contained 5 mM KCl and 3 mM of both $MgSO_4$ and ATP. Control was 1% ETOH; DES concentration was 10^{-4} M. Assay was initiated by adding ATP and DES (or ETOH) to the assay tubes, which were prewarmed 5 min at 38 C.

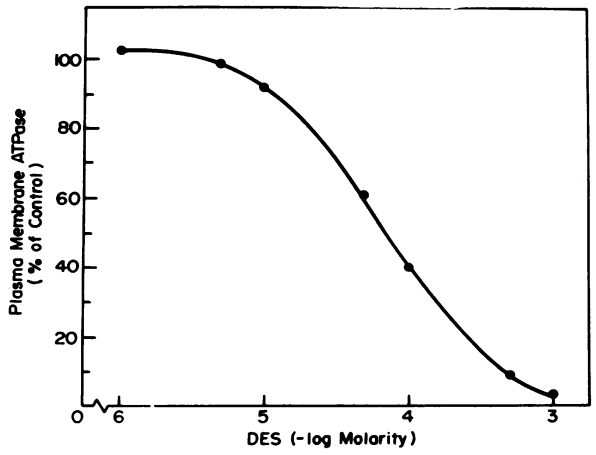

FIG. 2. Mg^{2+}-ATPase activity at various concentrations of DES. Assay medium contained 3 mM of both $MgSO_4$ and ATP. Activity of the control was 34.4 μmol Pi/mg pro

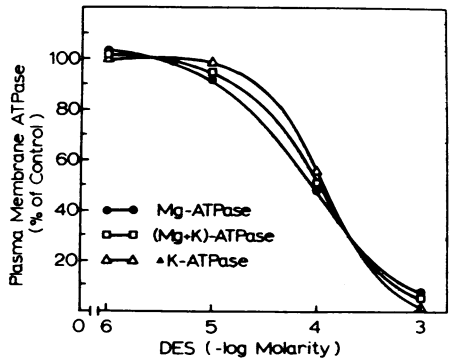

FIG. 3. Mg^{2+}-ATPase, (Mg^{2+}+K^+)-ATPase, and ΔK^+-ATPase activities at various concentrations of DES. Assay medium contained 3 mM of both $MgSO_4$ and ATP ± 50 mM KCl. Activities of the control were 52.2, 81.0, and 28.8 μmol Pi/mg protein·hr for Mg^{2+}-, (Mg^{2+}+K^+)-, and ΔK^+-ATPase, respectively.

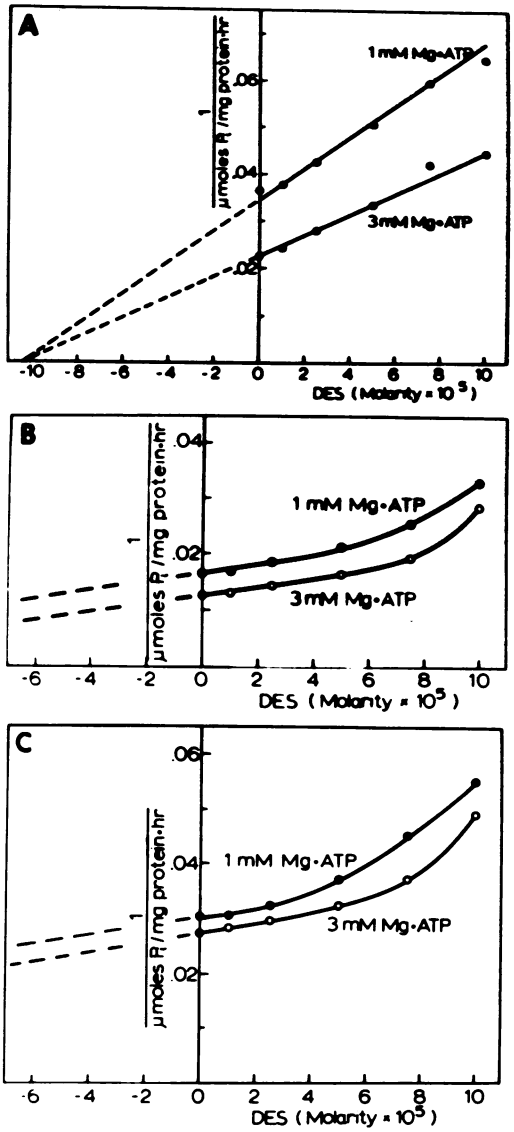

FIG. 4. Dixon plots of Mg^{2+}-ATPase (A), (Mg^{2+}+K^+)-ATPase (B), and ΔK^+-ATPase (C) activities in the presence of various concentrations of DES. All assay media contained either 1 mM or 3 mM of both $MgSO_4$ and ATP; 50 mM KCl was added for (B) and (C). ΔK^+-ATPase (C) was calculated from the data in (A) and (B). Straight lines (A) were drawn by linear regression ($r > 0.99$).

tion increased. The lines did not intersect, and thus, the inhibition was not typically competitive or noncompetitive.

However, plotting (Mg^{2+}+K^+)-ATPase activity *versus* the square of DES concentration produced straight lines with similar slopes at both concentrations of substrate (Fig. 5A). The same type of plot for ΔK^+-ATPase also produced straight lines with similar slopes (Fig. 5B).

Combining the Mg^{2+}-ATPase and (Mg^{2+}+K^+)-ATPase activities at 1 mM $MgSO_4$ and 1 mM ATP produced a Dixon plot with lines that intersected at -6.3×10^{-5} M (Fig. 6A). A Dixon plot of Mg^{2+}-ATPase and (Mg^{2+}+K^+)-ATPase at 3 mM $MgSO_4$ and 3 mM ATP (Fig. 6B) showed similar results except the distance between the $1/Mg^{2+}$-ATPase line and the $1/(Mg^{2+}+K^+)$-ATPase line was smaller. The lines intersected at -6.8×10^{-5} M, which was close to the intersection in Figure 6A. The significance of these kinetic data will be considered under "Discussion."

DES produced different effects on ATPase activity under various conditions. At the three concentrations of DES tested, 10^{-3}, 10^{-4}, and 10^{-5} M, the Triton-treated ATPase was less sensitive than the untreated ATPase to DES (Table I). In the absence of DES, the Triton-treated ATPase had about the same activity as the untreated enzyme. Also, treatment of oat roots with 10^{-4} M DES did not affect the ATPase activities of plasma membrane vesicles that were subsequently isolated from the treated roots (Table II).

Comparison of the inhibition of ATPase activities of the plasma membrane fraction in the presence of DES analogs (see ref. 3 for structures) showed that DES was the most inhibitory compound for all three ATPase activities (Table III). DES dipropionate decreased the ATPase activities only 10 to 15%. Hexestrol was intermediate between DES and DES dipropionate. Dienestrol did not inhibit at 10^{-4} M and perhapes even stimulated the Mg^{2+}-ATPase and the ΔK-ATPase slightly. Except for hexestrol, each analog affected all three ATPase activities to about the same degree. Hexestrol inhibited ΔK^+-ATPase less than (Mg^{2+}+K^+)-ATPase, and (Mg^{2+}+K^+)-ATPase less than Mg^{2+}-ATPase.

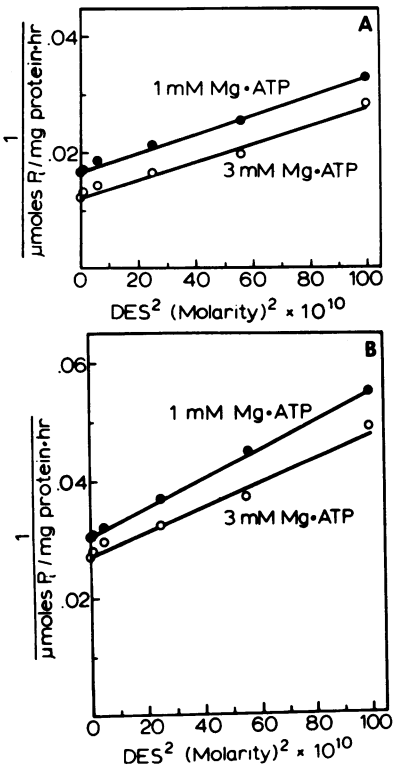

FIG. 5. Modified Dixon plots of (Mg^{2+}+K^+)-ATPase (A) and ΔK^+-ATPase (B) activities. Data are from Figure 4, B and C and are plotted as activity *versus* the square of the DES concentration. Lines drawn by linear regression ($r > 0.99$).

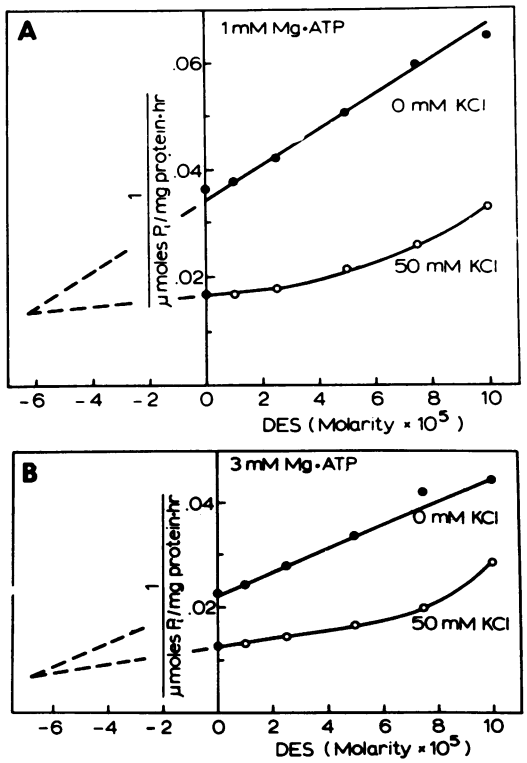

FIG. 6. Dixon plots of Mg^{2+}-ATPase and (Mg^{2+}+K^+)-ATPase activities in the presence of various concentrations of DES. Assay media contained either 1 mM (A) or 3 mM (B) of both $MgSO_4$ and ATP; 50 mM KCl added for (Mg^{2+}+K^+)-ATPase assays. Data are from Figure 4, A and B.

Table I. Comparison of Triton-treated and untreated ATPase activities in the presence of various concentrations of DES.

Treated plasma membrane vesicles were incubated in 0.05% Triton X-100 as described in "Materials and Methods." Assay media contained 3 mM of both $MgSO_4$ and ATP + 50 mM KCl. Activities of the controls were 39.7, 74.5, and 34.8 µmoles Pi/mg protein·hr for the untreated Mg^{2+}-, (Mg^{2+}+K^+)-, and ΔK^+-ATPase activities and 33.6, 68.6, and 35.0 for the corresponding Triton-treated ATPase activities.

Status of ATPase	DES Concentration (M)	ATPase Activity (% of Control) Mg^{2+}	Mg^{2+}+K^+	ΔK^+
Triton-treated or untreated Control (1% ETOH)	0	100	100	100
Untreated	10^{-5}	92	94	98
Triton-treated	10^{-5}	106	104	103
Untreated	10^{-4}	49	51	56
Triton-treated	10^{-4}	95	90	84
Untreated	10^{-3}	8	5	2
Triton-treated	10^{-3}	22	14	5

DISCUSSION

The fact that ΔK^+-ATPase activity was less sensitive than Mg^{2+}-ATPase activity between 10^{-5} and 10^{-4} M DES (Fig. 3) suggests that KCl protects the enzyme from inhibition by DES. The smaller slopes of the (Mg^{2+}+K^+)-ATPase and ΔK^+-ATPase at low DES concentrations (Fig. 4, B and C) compared to the larger slopes of the Mg^{2+}-ATPase activities (Fig. 4A) support this contention. Figure 6, A and B shows the differences in the slopes more clearly because the Mg^{2+}-ATPase and (Mg^{2+}+K^+)-ATPase are plotted on the same graph. This protection of the ATPase by KCl may be relevant since K^+ absorption by roots was inhibited less by DES

Table II. ATPase activities of the plasma membrane fraction isolated from roots treated with 10^{-4} M DES.

Roots (25 g) were incubated 5 min in 10^{-4} M DES (or 1% ETOH). After being rinsed with 5 changes of 500 ml 1% ETOH, the roots were homogenized, and the plasma membrane fraction was isolated as usual. Assay media contained 3 mM of both $MgSO_4$ and ATP ± 50 mM KCl. Activities of the control were 31.4, 54.2, and 22.8 μmoles Pi/mg protein·hr for Mg^{2+}-, (Mg^{2+}+K^+)-, and ΔK^+-ATPase, respectively.

Treatment	ATPase Activity (% of Control) Mg^{2+}	Mg^{2+}+K^+	ΔK^+
Control (% ETOH)	100	100	100
DES (10^{-4} M)	100	101	104

Table III. ATPase activities in the presence of 10^{-4} M DES analogs.

Assay media contained 3 mM of both $MgSO_4$ and ATP ± 50 mM KCl. Activities of the control were 46.8, 82.2, and 34.8 μmoles Pi/mg protein·hr for Mg^{2+}-, (Mg^{2+}+K^+)-, and ΔK^+-ATPase, respectively.

Compound (10^{-4} M)	ATPase Activity (% of Control) Mg^{2+}	Mg^{2+}+K^+	ΔK^+
Control (1% ETOH)	100	100	100
DES	50	51	54
Hexestrol	68	77	89
DES dipropionate	90	85	88
Dienestrol	109	99	103

as the K^+ concentration was increased (3). Also, K^+ influx may be insensitive to DES at high K^+ concentrations if a significant passive component of K^+ influx exists under those circumstances.

The kinetics of DES inhibition of (Mg^{2+}+K^+)-ATPase and ΔK^+-ATPase differed from those of Mg^{2+}-ATPase. DES inhibited the Mg^{2+}-ATPase noncompetitively (Fig. 4A) as it did pyruvate kinase (17) and glucose-6-P dehydrogenase (10). The K_i of DES for the Mg^{2+}-ATPase was 1.1×10^{-4} M; a higher value than the K_i = 1.5×10^{-5} M for (Na^++K^+)-ATPase or rat brain (23). However, inhibition of the (Mg^{2+}+K^+)-ATPase and ΔK^+-ATPase was more complex (Fig. 4, B and C). Similar kinetic data were reported for DES inhibition of glutamic dehydrogenase (30). The curved lines suggest that more than one DES molecule bound to each ATPase molecule in the presence of KCl to produce noncompetitive inhibition (28). Linearity between 1/(Mg^{2+}+K^+)-ATPase (or 1/ΔK^+-ATPase) and the square of DES concentration (Fig. 5, A and B) indicates that a minimum of two DES molecules bound to the enzyme in the presence of KCl. Also, Hill plots (18) (not shown) suggested that more than one DES molecule bound to the enzyme. K_i values are difficult to estimate in this situation and their validity is questionable (7, 28) because of the curvature of the (Mg^{2+}+K^+)-ATPase lines (Fig. 6); hence, they were not calculated.

The kinetic data clearly show that KCl affects the inhibition of the ATPase by DES. One possible explanation of this effect is that KCl displaces DES from the enzyme. However, in that instance KCl and DES should be competitors, but ΔK^+-ATPase is not competitively inhibited by DES (Figs. 4C and 5B). The binding of KCl to the enzyme may cause a conformational change which exposes another (or more than one) binding site for DES. This binding site may have a higher affinity for DES than the first site, but it is less inhibitory. The binding of DES to the second site results in a lower "effective" concentration of DES, and more DES must be added before the enzyme is inhibited. This conclusion is supported by the small slopes of the (Mg^++K^+)- and ΔK^+-ATPase lines near the origin (Fig. 4, B and C, and Fig. 6A and B). The induction of a conformational change by KCl may be important in the mechanism of the ATPase. Such K^+-induced conformational changes (22) have been proposed for the mechanism of the (Na^++K^+)-ATPase in animals (24). Thus, a conformational change induced by K^+ binding to the plasma membrane ATPase may result in K^+ transport across the plasma membrane in plant cells, also.

In addition to KCl, other factors also influence the DES inhibition. DES (10^{-4} M) inhibited the ATPase of prewarmed vesicles 89% (Fig. 1), but it inhibited the ATPase of vesicles not warmed before assay by only 60% (Fig. 2). This may be due to the membranes of prewarmed vesicles being more fluid (4), and hence DES may penetrate the lipid around the ATPase more readily (29). Also, this may be due to the movement of ions into the membrane or the lumen of vesicles during the warming of the vesicles (25). The distribution of ions may affect the structure of the membranes and the conformation of the ATPase, and hence DES binding to the ATPase.

Inhibition of the ATPase appears to be reversible. The ATPase of the plasma membrane fraction isolated from roots treated with DES had as much activity as vesicles isolated from controls (Table II). In contrast, inhibition of K^+ absorption was not reversed by a 2-min wash in 1% ETOH (3). However, the manipulation of the plasma membrane vesicles during the isolation procedure allowed more time for the DES to diffuse out of the membranes. DES inhibition of glucose-6-P dehydrogenase (10) and glutamic dehydrogenase (16) was reversible, also.

Inhibition of the ATPase by DES was reduced by treating the enzyme with Triton X-100. ATPase treated with Triton X-100 had about the same specific activity as the untreated enzyme, but DES did not inhibit the detergent-treated enzyme as much as the untreated control (Table I). The quality and quantity of lipids around ATPases affected the activity of several ATPases (5, 12, 21, 27). The lipid removed from the plasma membrane ATPase was probably replaced by Triton in order to keep the protein in suspension. The replacement of lipids by Triton possibly altered the conformation of the ATPase so DES could not bind as readily. Alternatively, the lipids may function directly in the binding of DES. Irrespective of the actual mechanism involved, it is clear that detergent treatment of the ATPase resulted in less inhibition by DES.

It is not just the lipid solubility of the DES molecule that accounts for the inhibition, however. DES dipropionate, which is more lipid-soluble than DES (3), inhibited the ATPase less than DES (Table III). It seems that the hydroxyl groups of DES are required for maximum inhibition. Also, steric interference by rigid ethyl side chains (dienestrol) can prevent the molecule from being inhibitory (Table III).

Comparison of the inhibition of (Mg^{2+}+K^+)-ATPase (Table III) and K^+ absorption (3) by DES analogs reveals that K^+ absorption is inhibited more than the (Mg^{2+}+K^+)-ATPase. The only exception is the equivalent inhibition of both activities by DES dipropionate. The sequence for inhibition of the ATPase is DES > hexestrol > DES dipropionate > dienestrol. The sequence for inhibition of K^+ absorption is DES > hexestrol > dienestrol > DES dipropionate. These data suggest that DES inhibits K^+ absorption by inhibiting the plasma membrane ATPase. Because K^+ absorption is inhibited more than the ATPase, possibly some other aspect of metabolism that is involved with absorption is inhibited, also. Reductions in ATP levels, ATPase activity, and K^+ absorption by DES are compared in the following paper (2).

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LITERATURE CITED

1. BALKE NE, TK HODGES 1977 Inhibition of ion absorption in oat roots: comparison of diethylstilbestrol and oligomycin. Plant Sci Lett 10: 319-325
2. BALKE NE, TK HODGES 1979 Comparison of reductions in adenosine triphosphate content, plasma membrane-associated adenosine triphosphatase activity, and potassium absorption in oat roots by diethylstilbestrol. Plant Physiol 63: 58-61
3. BALKE NE, TK HODGES 1979 Effect of diethylstilbestrol on ion fluxes in oat roots. Plant Physiol 63: 42-47
4. CHAPMAN D, DFH WALLACH 1968 Recent physical studies of phospholipids and natural membranes. *In* D Chapman, ed. Biological Membranes, Physical Fact and Function. Academic Press, New York, pp 125-202

5. DAHL JL, LE HOKIN 1974 The sodium-potassium adenosinetriphosphatase. Annu Rev Biochem 43: 327–356
6. DIXON M 1953 The determination of enzyme inhibitor constants. Biochem J 55: 170–171
7. DIXON M, EC WEBB 1964 Enzymes, Ed 2. Academic Press, New York, pp 312–331
8. DULLEY JR 1975 Determination of inorganic phosphate in the presence of detergents or protein. Anal Biochem 67: 91–96
9. DULLEY JR, PA GRIEVE 1975 A simple technique for eliminating interference by detergents in the Lowry method of protein determination. Anal Biochem 64: 136–141
10. DURHAM NN, LS ADAMS 1966 The structural specificity involved in the inhibition of a microbial D-glucose-6-phosphate dehydrogenase by synthetic estrogens. Biochim Biophys Acta 121: 90–101
11. FISKE CH, Y SUBBAROW 1925 The colorimetric determination of phosphorus. J Biol Chem 66: 375–400
12. HANSSON G, PJC KUIPER, A KYLIN 1973 Effect of preparation method on the induction of (sodium + potassium)-activated adenosine triphosphatase from sugar beet root and its lipid composition. Physiol Plant 28: 430–435
13. HEIKEL TAJ, GH LATHE 1970 The effect of 17 α-ethinyl-substituted steroids on adenosine triphosphatases of rat liver plasma membrane. Biochem J 118: 187–189
14. HODGES TK 1976 ATPases associated with membranes of plant cells. *In* U Lüttge, MG Pitman, eds, Encyclopedia of Plant Physiology, New Series, Vol 2, Part A. Springer-Verlag, New York, pp 260–283
15. HODGES TK, RT LEONARD 1974 Purification of a plasma membrane-bound adenosine triphosphatase from plant roots. Methods Enzymol 32: 392–406
16. KEMPFLE M, KO MOSEBACH, C BLANKE 1970 Influence on molecular weight and activity of bovine liver glutamate dehydrogenase (GluDH) by various effectors *in vitro*. II. Activity of GluDH and its subunits. Z Naturforsch 25B: 273–276
17. KIMBERG DV, KL YIELDING 1962 Pyruvate kinase: structural and functional changes induced by diethylstilbestrol and certain steroid hormones. J Biol Chem 237: 3233–3239
18. KOSHLAND DE JR 1970 The molecular basis for enzyme regulation. *In* PD Boyer, ed, The Enzymes, Ed 3 Vol I. Academic Press, New York, pp 342–396
19. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurements with the Folin phenol reagent. J Biol Chem 193: 265–275
20. MARTONOSI A 1968 Steroid effects on the calcium ion transport of rabbit skeletal muscle microsomes. Arch Biochem Biophys 125: 295–302
21. MARTONOSI A, J DONLEY, RA HALPIN 1968 Sarcoplasmic reticulum. III. The role of phospholipids in the adenosine triphosphatase activity and Ca^{2+} transport. J Biol Chem 243: 61–70
22. POST RL, S KUME, T TOBIN, B ORCUTT, AK SEN 1969 Flexibility of an active center in sodium-plus-potassium adenosine triphosphatase. J Gen Physiol 54: 306s–326s
23. ROBINSON JD 1970 Diethylstilbestrol—mode of inhibition of the ($Na^+ + K^+$)-dependent adenosine triphosphatase. Biochem Pharmacol 19: 1852–1855
24. SIEGEL GJ, RW ALBERS 1967 Sodium-potassium-activated adenosine triphosphatase of *Electrophorus* electric organ. J Biol Chem 242: 4972–4979
25. SZE H, TK HODGES 1976 Characterization of passive ion transport in plasma membrane vesicles of oat roots. Plant Physiol 58: 304–308
26. SZE, H, TK HODGES 1977 Selectivity of alkali cation influx across the plasma membrane of oat roots. Plant Physiol 59: 641–646
27. TANAKA R, T SAKAMOTO, Y SAKAMOTO 1971 Mechanism of lipid activation of Na, K, Mg-activated adenosine triphosphatase and K, Mg-activated phosphatase of bovine cerebral cortex. J Membr Biol 4: 42–51
28. WEBB JL 1963 Enzyme and Metabolic Inhibitors, Vol 1. Academic Press, New York, pp 149–191
29. WOOD A, LG PALEG 1974 Alteration of liposomal membrane fluidity by gibberellic acid. Aust J Plant Physiol 1: 31–40
30. YIELDING KL, GM TOMKINS 1960 Structural alterations in crystalline glutamic dehydrogenase induced by steroid hormones. Proc Nat Acad Sci USA 46: 1483–1488