# Specificity of Phospholipid Binding to Indole Acetic Acid and Other Auxins

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(Z. Naturforsch. **29 c**, 39–41 [1974]; received November 5, 1973)

Auxins, Phospholipids

Studies *in vitro* on the binding of phospholipids with IAA, α-NAA and some of their biologically inactive analogues demonstrate that the observed interaction between IAA and lecithin is not related to the primary action of the hormone in plant growth.

In recent years, Hertel *et al.*[1] have studied the primary binding sites of auxins. They pointed out that the binding site *in vitro* should be specific "*i. e.*, only growth-active transportable auxins, and competitive autiauxins or transport inhibitors but not inactive analogues should interact with the receptor." They further suggested that "auxin interacts with the plasma membrane at least during its transport and probably also during its action".

Since phospholipids are major constituents of the plasmalemma, a possible interaction between auxins and lecithin has been studied in recent years. Weigl[2–4] showed a strong physical association of auxins with lecithin: 1 mol lecithin was found to bind up to 0.8 mol IAA. New evidence that auxins interact with lecithin has been presented recently by Paleg *et al.*[5]. With nuclear magnetic resonance spectroscopy, they showed that there was a shift in the trimethylamino peak of lecithin after binding to IAA. Gibberellic acid exhibited a much weaker interaction.

The question of specificity of the observed phenomena remains a critical point in these studies. In an earlier paper[6], I concluded that stereo requirements necessary for biological activity were also required for polar auxin transport. α-NAA, biologically highly active, shows a high polarity in its transport, whereas α-DAA and β-NAA both biologically inactive (or nearly so), show no polarity.

*Abbreviations:* IAA, β-indoleacetic acid; α-NAA, α-naphthaleneacetic acid; β-NAA, β-naphthaleneacetic acid; α-DAA, α-decalylacetic acid; $CCl_4$, carbon tetrachloride; [$^{14}$C]Tryp, DL-[$^{14}$C]methylene-tryptophan; [$^{14}$C]MH, [$^{14}$C]2,3 maleic hydrazide; [$^{14}$C]Glc, D-[$^{14}$C]glucose.

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A possible interaction between these three substances labelled with $^{14}$C and phospholipids was studied *in vitro* as described by Weigl[2].

## Experimental

Distilled water (5 ml), in which an auxin or other substance was dissolved, was vigorously shaken for 2 min with 2 ml $CCl_4$ or light petroleum (b. p. 40 – 60 °C), in which phospholipids were dissolved. After centrifuging at $2700 \times g$ for 5 min, samples of 100 µl were taken from the water and the organic solvent layer. The radioactivity in both samples was counted in a liquid scintillation spectrometer; quench correction was done by internal standardization.

In addition to the auxins and its analogues, some other compounds chemically unrelated to plant hormones were tested. The results, expressed as nmoles of the radioactive substance in 5 ml water or 2 ml $CCl_4$ or 2 ml light petroleum, are presented in the tables.

## Results and Discussion

The data of Table I show that, in agreement with Weigl's observation, lecithin dissolved in $CCl_4$ moved

Table I. Interaction between lecithin * in $CCl_4$ (20 µmol/2 ml) and auxins and some of their analogues.

| Labelled compound ** | Amount 5 ml sample [nmol] | $CCl_4$ without lecithin | | $CCl_4$ with lecithin | |
|---|---|---|---|---|---|
| | | $H_2O$ | $CCl_4$ | $H_2O$ | $CCl_4$ |
| [1-$^{14}$C]IAA | 6.5 | 6.0 | 0.1 | 2.2 | 4.0 |
| [$^{14}$C]Tryp | 5.4 | 5.2 | 0.0 | 5.2 | 0.2 |
| [1-$^{14}$C]α-NAA | 4.0 | 3.7 | 0.2 | 0.6 | 3.9 |
| [1-$^{14}$C]α-DAA | 4.4 | 0.7 | 3.9 | 0.2 | 4.4 |

* Merck purest egg lecithin, Darmstadt.

** All labelled compounds (except α-DAA and β-NAA) were obtained from Radiochemical Centre, Amersham.

IAA out of an aqueous phase. Tryptophan did not react, whereas α-NAA behaved in the same way as IAA. The distribution of α-DAA mostly into the organic solvent obscured the results. I therefore dissolved lecithin in light petroleum in with α-DAA was less soluble. The data are presented in Table II.

Table II shows that some substances were strongly associated with lecithin and others were not. But there seemed to be no relation with the biological activity of the substances.

Table II. Interaction between egg lecithin dissolved in light petroleum (Petr., b. p. 40—60 °C) (20 μmol/2 ml) and a number of different substances.

| Labelled compound | Amount 5 ml sample [nmol] | Petr. without lecithin | | Petr. with lecithin | |
|---|---|---|---|---|---|
| | | $H_2O$ | Petr. | $H_2O$ | Petr. |
| [1-$^{14}$C] IAA | 6.5 | 6.0 | 0.0 | 3.4 | 4.2 |
| [$^{14}$C] Tryp | 5.4 | 5.4 | 0.0 | 5.4 | 0.1 |
| [1-$^{14}$C] α-NAA | 4.0 | 4.0 | 0.0 | 1.2 | 3.2 |
| [1-$^{14}$C] α-DAA | 4.4 | 3.9 | 0.6 | 0.4 | 4.5 |
| [$^{14}$C] MH | 5.3 | 5.3 | 0.0 | 5.3 | 0.0 |
| [$^{14}$C] Glc | 51.6 | 50.7 | 0.0 | 51.0 | 0.2 |
| [1-$^{14}$C] α-NAA | 0.40 | 0.41 | 0.00 | 0.13 | 0.37 |
| [1-$^{14}$C] β-NAA | 0.63 | 0.60 | 0.02 | 0.10 | 0.53 |
| [1-$^{14}$C] α-DAA | 0.44 | 0.38 | 0.03 | 0.02 | 0.47 |

Weigl [4] stated further that the binding of auxin to phospholipids was established mainly by a complementary binding between the charged groups of the two molecules. In accord with this hypothesis, he showed a weaker interaction with cephalin and with phosphatidylserine compared to lecithin. To test his hypothesis, I investigated the binding of β-IAA (with a fractional positive charge in the nucleus) and α-NAA (a weak positive charge, if any, in the nucleus) to lecithin and to cephalin. These data are presented in Table III.

Table III. Interactions of synthetic lecithin * and cephalin * with auxins.

| Labelled compound | Amount 5 ml sample [nmol] | $CCl_4$ without phospholipid | | $CCl_4$ with phospholipid | |
|---|---|---|---|---|---|
| | | $H_2O$ | $CCl_4$ | $H_2O$ | $CCl_4$ |
| | L-α-Lecithin (40 μmol/2 ml) | | | synth. | purum |
| [1-$^{14}$C] IAA | 5.3 | 5.2 | 0.0 | 3.0 | 1.8 |
| [1-$^{14}$C] α-NAA | 18.0 | 16.0 | 2.0 | 7.4 | 9.2 |
| | L-α-Cephalin (40 μmol/2 ml) | | | synth. | purum |
| [1-$^{14}$C] IAA | 5.3 | 5.3 | 0.0 | 5.1 | 0.1 |
| [1-$^{14}$C] α-NAA | 18.0 | 16.1 | 2.1 | 10.7 | 6.8 |

* From Fluka AG, Buchs.

Table III shows that IAA had a much weaker affinity to cephalin as to lecithin, whereas α-NAA was strongly associated with both phospholipids. These observations cannot be explained by a simple complementary binding between differently charged groups, as suggested by Weigl [4].

Lastly, different amounts of synthetic lecithin were dissolved in $CCl_4$ to check Weigl's hypothesis of a 1 : 0.8 binding ratio of lecithin to IAA. Fig. 1

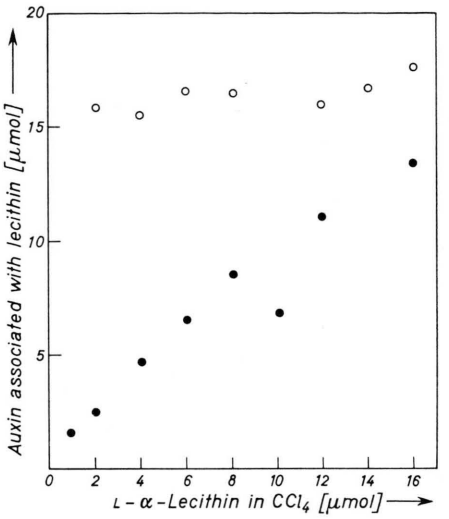


Fig. 1. Binding of IAA (●) and of α-NAA (○) to variable amounts of lecithin. From both auxins 20 μmol was dissolved in the water fraction.

confirms Weigl's finding for IAA. α-NAA did not show such a relationship at all. Even with relatively low concentrations of lecithin, 80% of the total number of NAA molecules in the water fraction was associated with the lecithin in the $CCl_4$. Hence, α-NAA reacts with phospholipids in a way which is completely different from that suggested for IAA by Weigl [4].

There is strong evidence that IAA and α-NAA move in the same polar transport system (Leopold and Lam [7]; Hertel *et al.* [8]) and that both auxins behave essentially the same in binding kinetics to a homogenate from corn coleoptiles (Hertel *et al.* [1]). Therefore it is likely that both auxins act on the same site for growth.

Conclusions:

1. α-NAA and a number of biologically inactive analogues show a pronounced association with lecithin.

2. The binding of α-NAA to phospholipids is characteristically different from that of IAA.

3. The observed binding of the plant hormone IAA to lecithin is not related to the primary action of the hormone in plant growth. As a hypothesis I would like to suggest that it seems more likely that proteins play a role in the reception of the auxin molecule at the outer membrane (Osborne and Mullins [9]).

The author is indebted to Mr. J. C. Rigg (Centre for Agricultural Publishing and Documentation, Wageningen) for correction of the English text.

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