description previously
detailed in Fig. 1. Four major peaks of
radioactivity were observed. The initial peak (i.e. at the
front peak represents incorporation of label into neutral
lipids. It can clearly be seen that in the presence of dexa-
methasone inhibition of incorporation was observed over a
range of dexamethasone concentrations (data not shown)
with a 50% inhibition observed with 7x10^{-6} M-dexamethasone.

In experiments in which the incorporation of radioactive sugars
other than glucosamine was measured, it was observed that a comparable profile of incorporation was observed. When radiolabelled galactose was utilized, incorporation of label into lipids was inhibited by dexamethasone in a dose-dependent manner identical to that observed for glucosamine. However, in experiments utilizing radiolabelled galactosamine, the degree of inhibition of incorporation was significantly less than that observed with glucosamine or galactose (e.g. 10^{-6} M-dexamethasone caused only 20–30% inhibition of galactosamine incorporation).

In further experiments, incorporation of all three radioactive sugars into lipids was measured over time periods from 0 to 24 h. Throughout this time period the percentage incorporation of radioactively labelled sugars into specific lipids remained the same. Dexamethasone produced an equivalent inhibition of incorporation at all time points studied, without modification of the lipid profile.

In parallel experiments a permissive interaction was clearly observed between dexamethasone (10^{-7} M) and insulin (10^{-9} M) with regard to the effect of insulin on hepatocyte conversion of [U-14C]glucose to glycogen. In the absence of dexamethasone insulin stimulated the conversion by 118%, while in the presence of dexamethasone (10^{-7} M) insulin caused a 181% stimulation.

The mechanism by which dexamethasone inhibits incorporation of radioactively labelled sugars into glycolipids may result from inhibition of sugar uptake or modification of intracellular processing of sugars. Our data indicate that the permissive interaction between glucocorticoids and insulin may not relate to increased synthesis of membrane glycospholipids. However, this does not indicate that glycospholipids are not involved in insulin signal transduction. Experiments to determine the detailed mechanism of the effect of dexamethasone are currently under way.

I would like to thank the Juvenile Diabetes Foundation for their financial support.


Received 27 November 1989

Co-ordination of chromophore–apoprotein synthesis in the developing leaf of Arena sativa L.

A. P. BENNETT, T. G. E. DAVIES,† H. THOMAS† and L. J. ROGERS*†

*Department of Biochemistry, University College of Wales, Aberystwyth, Dyfed SY23 3DD, Wales, U.K. and †Plant and Cell Biology Department, A.F.R.C. Institute of Animal and Grassland Production, Aberystwyth, Dyfed SY23 3EB, Wales, U.K.

Gabaculine (3-amino 2,3-dihydrobenzoic acid) inhibits tetrapyrrrole biosynthesis in higher plants and has been shown to prevent formation of chlorophyll [1], the chromophore prosthetic group of phytochrome [2] and extrachloroplastic haem [3]. Inhibition has been shown to occur at the final step, catalysed by glutamate-1-semialdehyde aminotransferase [4], in the so-called C-5 pathway to 5-aminovaleric acid [5], which is the crucial precursor to tetrapyrrrole formation. Previous studies investigating inhibition of plant tetrapyrrrole synthesis have with few exceptions utilized leaf segments, excised leaves or tissue slices; however, gabaculine is readily taken up through the roots of intact seedlings and the advantage of being able to investigate the metabolic effects of inhibition under near-normal physiological conditions in vivo has been exploited in the studies described here.
Plants of *Avena sativa* L. were grown with an 8 h photoperiod at 20°C for about 2 weeks until the observed emergence of the third leaf from its sheath. Gabaculine was dissolved in the hydroponic growth medium to a concentration of 50 μM; control plants were maintained in the same growth conditions except for the omission of inhibitor, and all plants were grown for a further 7 days before harvesting. The third leaf was dissected from the surrounding leaves and divided into segments; eight leaves each from control and treated plants were used.

Gabaculine-grown plants exhibited no obvious inhibition of growth relative to the control, even though the third leaf was distinctly etiolated in appearance. At this stage in their growth, third leaves from both plants were typically 23–25 cm in length. All the leaves selected for dissection were of uniform length (24 cm) and were dissected into 8 × 3 cm segments numbered from base upwards; corresponding sections were bulked and determinations of chlorophyll and carotenoids, soluble and particulate proteins and fresh weight were performed.

Fresh weights were virtually identical for corresponding sections along both treated and untreated leaves, whereas at 50 μM-gabaculine the amounts of total chlorophyll (Fig. 1) were greatly decreased along the length of the leaf, though less so at the leaf tip where chloroplasts were already present before exposure to gabaculine. A large decrease in total carotenoid levels was also evident (Fig. 1). Protein determinations along the leaf indicated a decrease in the levels of detectable particulate proteins in treated plants, while amounts of soluble protein appeared to be unaffected and were possibly even slightly increased in the presence of gabaculine (data not shown). It is likely that the massive inhibition of chlorophyll formation in gabaculine-treated plants prevents the normal assembly of thylakoid membranes resulting in the observed decrease in particulate protein.

Additional analyses of extracted proteins was performed by native and denaturing PAGE, coupled with Western blotting using antibodies to LHCP-2 and cytochrome f, while amounts of extrachloroplastidic haem were investigated by activity staining of haem after SDS/PAGE of the soluble fractions.

A significant decrease in LHCP-2 was observed in inhibitor-grown plants; this paralleled the decrease in chlorophyll indicating a close co-ordination in synthesis of protein and prosthetic group. In contrast, these same leaves the amounts of haem and of cytochrome f protein were largely unaffected by the presence of gabaculine. Thus it would appear that cytochrome f formation continued in conditions which had caused marked inhibition of chlorophyll and its associated proteins, even though both prosthetic groups originate from the same precursors. Identical extractions and measurements were performed on the second leaves from the same plants — these were fully green before gabaculine treatment and no decrease in any of the factors considered was observed in treated plants. It is not clear from this investigation whether this is due to a lack of uptake of the inhibitor in the established leaves or to a slow turnover of proteins in these leaves, though the latter explanation is favoured.

Polyclonal Antibodies Limited, Dyfed, Wales, is thanked for collaboration in the production of antibodies to LHCP-2 and cytochrome f.


Received 24 November 1989

An improved purification procedure for uroporphyrinogen III synthase from *Euglena gracilis*

NICOLA J. GUMPEL and ALISON G. SMITH
Botany School, University of Cambridge, Downing St, Cambridge CB2 3EA, U.K.

The overall pathways for the biosynthesis of haem, chlorophyll and vitamin B_{12} are well known and are essentially the same in all organisms [1]. The first cyclic tetrapyrrole in porphyrin synthesis is uroporphyrinogen III (urogen III). This is synthesized from the monopyrrole porphobilinogen (PBG) by the sequential action of two enzymes, hydroxymethylbilane synthase (HMBS, EC 4.3.1.8., also known as PBG deaminase) and urogen III synthase (EC 4.2.1.75, previously called cosynthetase). HMBS catalyses the head-to-tail condensation of four molecules of PBG with elimination of ammonia to form the unstable linear tetrapyrrole, HMB. In the absence of the second enzyme, the non-metabolizable isomer urogen I is formed. Urogen III synthase catalyses the ring closure with concomitant rearrangement of the D ring to give urogen III. These enzymes are present in all cells at 'housekeeping' levels, but must be highly expressed in tissue such as erythrocytes and in the chloroplasts of young

Abbreviations used: PBG, porphobilinogen; HMBS, hydroxymethylbilane synthase; urogen, uroporphyrinogen.