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Association–Dissociation and Abnormal Kinetics of Bovine α-Acetylgalactosaminidase*

Ching-Te Wang† and Bernard Weissmann‡

ABSTRACT: Gel filtration and kinetic studies show that bovine α-acetylgalactosaminidase exists in dilute solutions as a mixture of active oligomer(s) and inactive monomer. The rapidly reversible equilibrium between these species is temperature dependent and is linked with binding of substrate or competitive inhibitor. Association is enhanced by increased enzyme concentration, increased substrate concentration, or decreased temperature.

The most associated species has a molecular weight of about 155,000 and the monomer has a molecular weight between 30,000 and 42,000. These findings account for a previously observed kinetic abnormality, the decrease of apparent specific activity on dilution, now shown to apply also to the corresponding enzymes of the rabbit, turtle, and frog, but not those of the pig, rat, guinea pig, chicken, fish, or earthworm.

Unusual kinetic behavior was found earlier for a partially purified preparation of α-acetylgalactosaminidase from beef liver, using phenyl *N*-acetyl-α-galactosaminide as a test substrate (Weissmann and Hinrichsen, 1969). The phenomena observed included a decline in apparent specific activity of the enzyme on dilution. This abnormality was most prominent at low substrate concentrations and higher temperatures. The rapidly reversible dissociation of an active enzyme into inactive subunits was offered as a possible, but unproven, explanation.

Parallel physical and kinetic observations now demonstrate such a facile association–dissociation, which is linked with binding of substrate or competitive inhibitor. This enzyme accordingly affords an illustration of the interaction of "ligand" binding[1] with protein polymerization (Klapper and Klotz, 1968; Frieden, 1967; Nichol *et al.*, 1967) apparently simpler than most of the numerous well documented cases presently known, which generally involve interaction of complex regulatory enzymes with allosteric effectors (Frieden and Colman, 1967; Vagelos *et al.*, 1963; Numa *et al.*, 1967; Datta *et al.*, 1964; Hirata *et al.*, 1965; Scrutton and Utter, 1965; Iwatsuki and Okazaki, 1967; Maley and Maley, 1968; LeJohn *et al.*, 1969; DeVincenzi and Hedrick, 1970; Constantinides and Deal, 1970; Leary and Kohlaw, 1970; Long *et al.*, 1970).

Experimental Section

Materials. The beef liver α-acetylgalactosaminidase preparation whose kinetic and chromatographic behavior form the subject of this work was the partially purified specimen described earlier (Weissmann and Hinrichsen, 1969). Its activity had not changed significantly during storage for

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[1] For present purposes, "ligand" has reference only to binding of small molecules and disregards protein–protein binding, which is referred to explicitly.

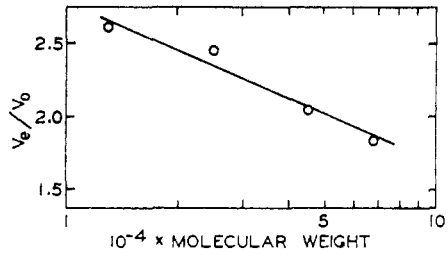

FIGURE 1: Semilog plot, summarizing the chromatography of some nondissociating proteins on Sephadex G-150 in the frontal analysis mode. The proteins and the molecular weight values plotted are: cytochrome *c* (13,000), α-chymotrypsinogen A (25,000), ovalbumin (45,000), and bovine serum albumin (68,000). The column was operated at 20° and Blue Dextran was used to establish the void volume. For other details see text.

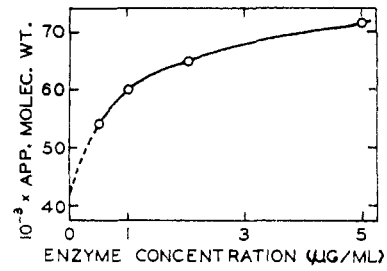

FIGURE 2: Apparent molecular weight values at 38° for bovine α-acetylgalactosaminidase at various concentrations of partially purified preparation. The values plotted were determined by chromatography on Sephadex G-150 in the frontal analysis mode and the enzyme concentrations represent plateau values in the column eluates.

1–2 years at −18°. Beef, pig, rat, guinea pig, and rabbit tissues used were frozen shortly after death of the animals. Frozen fresh frog (*Rana pipiens*) and turtle (*Chelydra serpentina*) liver were obtained from Pelfreeze Biologicals, Inc., fresh pike perch (*Stizostedium vitreum*) and fresh frozen chicken liver from local markets, and live earthworms (*Lumbricus terrestris*) from the Lemberger Co. Cytochrome *c* (type VI from horse heart), egg albumin (grade V), α-chymotrypsinogen A (type II from bovine pancreas), and crystalline bovine serum albumin were purified proteins from the Sigma Chemical Co. Urea was an enzyme grade reagent from Mann Research Laboratories. Blue Dextran and Sephadex G-150 (40–120 μ) were products of Pharmacia Fine Chemicals, Inc. The preparation of *p*-nitrophenyl *N*-acetyl-α-D-galactosaminide has been described (Weissmann, 1970).

In the preparation of extracts for the comparative survey of abnormal kinetics of α-acetylgalactosaminidase from various tissues, homogenates of the frozen tissues with three volumes of water were centrifuged. Aliquots of the supernatant extracts were assayed for α-acetylgalactosaminidase. Ammonium sulfate fractions of the extracts, collected between 0.20 and 0.60 saturation, were dialyzed and preserved at −18° for use in kinetic experiments.

General Procedures. Thermometers were compared to a secondary standard certified by the National Bureau of Standards.[2] Buffers used for enzyme dilution and assay were regularly supplemented with 0.1–0.5 mg/ml of albumin, to avoid enzyme inactivation. Enzyme assays were generally performed, as previously (Weissmann and Hinrichsen, 1969), with 10 mM phenyl *N*-acetyl-α-D-galactosaminide at 38° for 1 hr in 0.05 M sodium citrate buffer of pH 4.7 (pH 5.0 for column eluates). Column eluates containing phenyl glycoside were assayed with 1 mM *p*-nitrophenyl *N*-acetyl-α-D-galactosaminide at 38° for 1 hr at pH 5.0. Column eluates were monitored for albumin and chymotrypsinogen at 280 mμ, for cytochrome *c* at 412 mμ, for Blue Dextran at 660 mμ, and for α-acetylgalactosaminidase by assays of enzymic activity.

Gel Filtration. Columns of Sephadex G-150 (1.3 × 39 cm) were poured and settled by washing with buffer at their highest temperature of use. For washing and for development, 0.05 M sodium citrate buffer (pH 5.0) was used, supplemented in enzyme chromatograms by 0.1 mg/ml of albumin and, where so specified, by phenyl *N*-acetyl-α-D-galactosaminide (5 mM) or *N*-acetylgalactosamine (5 mM). The eluate was collected in fractions of about 1 ml, precisely measured by weighing, which were analyzed for enzyme, Blue Dextran, or standard proteins, as required.

In frontal analysis experiments (Winzor and Scheraga, 1963, 1964), run at 38°, a solution of the column load in 25 ml of development buffer was applied to the top of the drained column, which had been previously washed with developer. The volume for elution, V_e, of the advancing front at half-height was estimated graphically from the analyses of effluent fractions. The void volume, V_0, was checked daily with Blue Dextran. Values of apparent molecular weight, M_W, were assigned, based on calibration with cytochrome *c* and bovine serum albumin under conditions of use, assuming a linear relationship between V_e/V_0 and log M_W (Henn and Ackers, 1969; but see Winzor and Scheraga, 1963). Frontal analysis experiments with proteins of known molecular weight (Figure 1) indicate that such a linear relationship is at least roughly applicable.

In peak migration experiments (Andrews, 1965; Leach and O'Shea, 1965), the load was applied as a solution in 1 ml of developer containing a small quantity of Blue Dextran and 10% of sucrose. The effluent volume for the protein peak was taken as V_e and the volume for the Blue Dextran peak as V_0. A single column was used for consecutive measurements made at 38, 25, and 4°; the measurements at 32° were made on a second column. Values of M_W were estimated, based on calibration at each temperature of use with Blue Dextran, cytochrome *c*, and bovine serum albumin, exploiting the linear relationship between V_e/V_0 and log M_w, well documented for this mode of operation.

For given conditions in either mode of operation, the average day to day variation of V_0 was about ±1.2% and a similar variation in V_e might be expected. Analytical errors of this magnitude, if they were noncompensating, would produce a variation in M_W of ±3% at M_W of 160,000, and ±4% at M_W of 40,000.

Results

Variations of Apparent Molecular Weight. The results of gel filtration experiments at 38° by the frontal analysis method, expressed as apparent molecular weight, are summarized in Figure 2. It is clear that the apparent molecular weight of the enzyme decreases regularly with dilution, as anticipated for a dissociating system. Values between 30,000 and 42,000 are estimated for the molecular weight of the

[2] The temperatures reported earlier (Weissmann and Hinrichsen, 1969) were slightly in error. A temperature reported earlier as 42° corresponds to a corrected value of 41.2, 38–37.1, 32–31.6, and 25–24.9°.

TABLE I: Apparent Molecular Weight of Bovine α-Acetylgalactosaminidase, Estimated from Gel Filtration Data.[a]

Temp (°C)	Addn to Developer	V_e/V_0	App Mol Wt
38	None	1.56	52,000
38	GalNAc	1.46	70,000
32	None	1.66	61,000
32	Substrate	1.46	105,000
32	GalNAc	1.43	114,000
25	None	1.51	74,000
4	None	1.38	130,000
4	GalNAc	1.33	155,000
4	GalNAc	1.33	155,000[b]

[a] The enzyme was chromatographed in the peak migration mode on calibrated columns of Sephadex G-150, as described in the Experimental Section, and monitored in the eluate by enzyme assays. The developer, bovine serum albumin (100 μg/ml) in buffer, was supplemented with 5 mM *N*-acetylgalactosamine or 5 mM phenyl *N*-acetyl-α-galactosaminide (substrate), where so indicated. [b] Enzyme concentration 4 μg/ml (peak value in eluate) in this experiment only; 0.4 μg/ml in all others.

most dissociated form (monomer) from extrapolation of the data to zero enzyme concentration.

The influence of other factors on dissociation is illustrated by the results shown in Table I, which were obtained from peak migration experiments. That a profound increase in degree of association occurs as the temperature is decreased can be seen from the measurements made in the absence of substrate or inhibitor. Preliminary experiments indicated that association was promoted either by substrate or by *N*-acetylgalactosamine and that the latter substance behaved as a competitive inhibitor (Figure 3). From the results of gel filtration experiments at 32° shown in Table I, it can be seen that substrate and inhibitor increase degree of association to a roughly comparable extent at 32° under the conditions tested. Qualitatively similar results are seen for inhibitor at 38 and 4°. The largest value of molecular weight found was at 4° in presence of inhibitor. This value of about 155,000 is believed to represent the molecular weight of the most aggregated species, since no further change is found when the enzyme concentration is increased tenfold. Based on the numerical values of molecular weight estimated for the most dissociated and most associated species, the latter will for convenience be referred to hereafter as a tetramer, but with the understanding that the present data do not discriminate satisfactorily between this and a pentamer, or perhaps even a hexamer.

Specific Activity and Enzyme Concentration. As seen in Figure 4, the dependence of specific activity on enzyme concentration is that expected for dissociation of active oligomer to relatively inactive or completely inactive monomer. Discrimination between these possibilities is uncertain because the considerable analytical inaccuracy of the lowest assay values limits the precision of extrapolation to zero enzyme concentration (100% monomer). Although methodological difficulties obstruct the precise correlation of the physical and the kinetic findings, the data of Table I suggest a

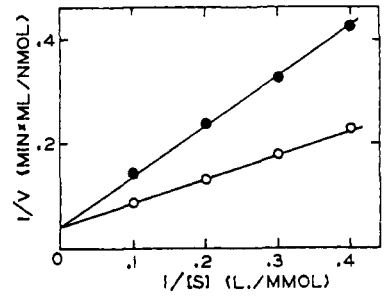

FIGURE 3: Lineweaver–Burk plot showing inhibition of bovine α-acetylgalactosaminidase by *N*-acetylgalactosamine. The measurements were made with digests of phenyl *N*-acetyl-α-galactosaminide and enzyme (1 μg/ml) at 33° and pH 4.7 in presence (●) and absence (○) of 10 mM *N*-acetylgalactosamine (K_M, 10.8 mM; K_i, 8.7 mM; from data shown).

considerable proportion of tetramer exist in the presence of substrate at 32° and higher enzyme concentrations, where the apparent specific activity is seen in Figure 4 to approach a plateau value. It may be concluded that tetramer must be at least as active, perhaps far more active, than any other oligomeric species which may be present. Nothing can be inferred otherwise regarding the enzymic activity of dimer or trimer, or regarding the prevalence of such species in equilibria.

As shown in previous work (Weissmann and Hinrichsen, 1969), the relationship between steady-state velocity and enzyme concentration over a restricted range of enzyme concentration fits the empirical relationship: velocity = constant × [enzyme]^n. Deviations from the value of unity for the exponent n, which was estimated from log–log plots, were used as measures of a nonlinear relationship between velocity and enzyme concentration. In log–log plots (not shown) of the data of Figure 4, consideration will show the instantaneous slope of the curves at any point to have the value $n - 1$. From such plots, for 32° n is estimated to be 1.05 at 5 μg/ml, corresponding to an almost linear relationship, increasing to 1.37 at 1 μg/ml; for 38° n is 1.19 at 5 μg/ml and 1.47 at 1 μg/ml. Nonlinearity is thus enhanced at lower enzyme concentrations. A limiting value for n of 1.50 was

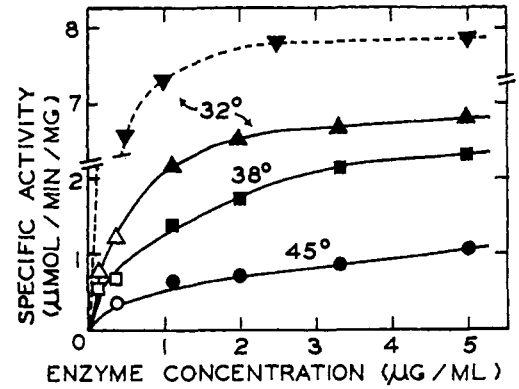

FIGURE 4: Variation of apparent specific activity with concentration for partially purified bovine α-acetylgalactosaminidase. Results are shown for 1 mM substrate at 45° (○,●), 38° (□,■), and 32° (△,▲), and for 5 mM substrate at 32° (▼). The extrapolation to the origin is arbitary (see text). Fixed intervals of 24 min (shaded symbols) and 2 hr (open symbols) were used for the assays with phenyl glycoside in 0.05 M sodium citrate buffer of pH 5.0.

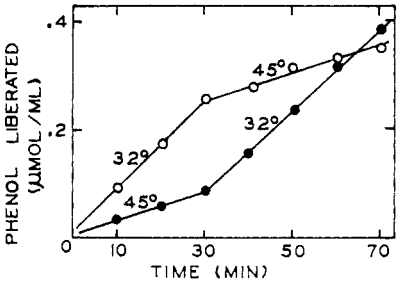

FIGURE 5: Progress curves for two identical digests, initially at 32 and 45°, of phenyl *N*-acetyl-α-galactosaminide (5 mM) with bovine α-acetylgalactosaminidase (1 μg/ml) at pH 4.7. After sampling at 30 min, the digest tubes were interchanged in their water baths and the measurements were continued.

assumed in attempting extrapolation to zero enzyme concentration (Figure 4).

Reversibility of Temperature Effects. In experiments summarized in Figure 5, progress curves were measured for the same digest consecutively, first at a higher, then at a lower temperature. In a second identical digest the temperature sequence was reversed. The almost equal slopes of progress lines for the same temperatures in the two digests show essentially complete reversibility of the temperature-induced changes in specific activity, which, as made clear by the preceding experiments, must stem from association and dissociation.

Rapidity of Association and Dissociation. When cold concentrated enzyme is mixed rapidly with a large volume of substrate solution maintained at 38°, an initially high reaction velocity declines in some 0.5–1.0 min to a lower steady-state value. This phenomenon, earlier detected as a "small initial burst" (Weissmann and Hinrichsen, 1969), is now attributed to dissociation of active enzyme on dilution and temperature increase. Isothermal association of the enzyme can be demonstrated on addition of substrate, and it has a time constant of similar magnitude to that for dissociation, as seen in Figure 6.

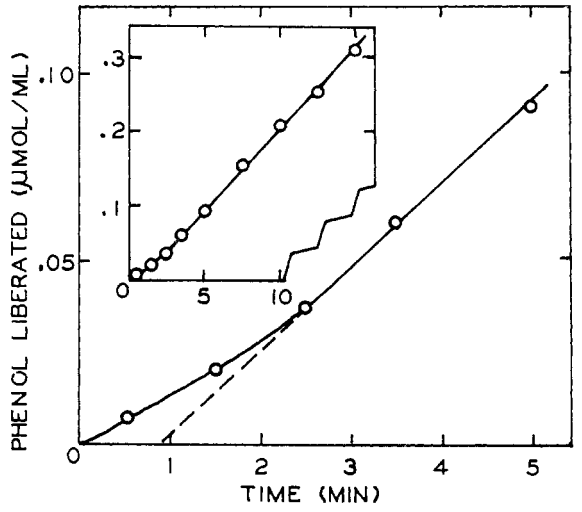

FIGURE 6: Two views of the progress curve for a digest initiated at 32° by rapidly mixing 9 ml of bovine α-acetylgalactosaminidase (3.3 μg/ml; final concentration, 2.5 μg/ml) and 3 ml of 20 mM phenyl *N*-acetyl-α-galactosaminide (in buffer of pH 4.7). The broken line is an extension of the steady-state, linear portion of the progress curve, best seen in the inset.

TABLE II: Abundance and Kinetic Abnormality of α-Acetylgalactosaminidase in Various Tissues.[a]

Tissue	Activity (Units/g)	Exponent *n*
Beef liver[b]	0.69	1.24
Beef spleen	0.12	1.18
Beef kidney	0.51	1.24
Rabbit liver	1.06	1.35
Frog liver	0.31	1.32
Turtle liver	0.03	1.22
Pig liver	(Lost)	(1.00)
Rat liver	0.39	(1.00)
Guinea pig liver	0.46	(1.00)
Chicken liver	0.50	(1.00)
Fish liver	0.41	(1.00)
Earthworm extract	1.22	(1.00)

[a] Results of standard assays of centrifuged homogenates at 38° and pH 4.7 with phenyl *N*-acetyl-α-galactosaminide have been expressed as activity per gram of moist tissue. For each tissue, a dialyzed crude ammonium sulfate fraction has also been assayed at 38° at a concentration corresponding to about 10 mU/ml and at three lesser concentrations. The results have been fitted (Weissmann and Hinrichsen, 1969) to the empirical relationship: velocity = constant × [enzyme]^n and the values of the exponent *n* so determined are given. Where *n* is listed as unity, the relationship is linear within experimental error. [b] Two other specimens of beef liver gave similar values of *n*. For other tissues, only a single specimen was examined.

Urea and Activity. Urea powerfully inhibits the enzyme (51% inhibition in 1 M, 82% in 2 M, and 96% in 3 M urea; at 38° and pH 5.0 in the presence of 1 mg/ml of bovine serum albumin). Some 80% of the original activity is still demonstrable on dilution of a solution of enzyme in 3 M urea, but only 40% on dilution of a 4 M urea solution. Presumably, urea inhibits at low concentrations by promoting reversible dissociation of enzyme into inactive monomer, with irreversible dissociation of monomer into subsubunits at higher concentrations of urea (*cf.* Frieden, 1963; DeVincenzi and Hedrick, 1970).

Species Variation of Kinetic Abnormality. Since, in earlier work, the specific activities of pig or rat liver enzymes did not show the concentration dependence found for the beef liver enzyme, a number of tissues were surveyed. As seen in Table II, the kinetic abnormality is detectable in enzymes from beef spleen or kidney, from several specimens of beef liver, from rabbit, frog, or turtle liver, but not from tissues of the pig, rat, guinea pig, chicken, fish, or earthworm. Thus the kinetic abnormality, presumably an indicator of enzyme dissociation, shows species variation but not individual or tissue variation. No simple evolutionary pattern is obvious in the results.

Discussion

As already mentioned, the designation of tetramer for the most aggregated species of enzyme is only an approximation, subject to the errors inherent in the empirical gel filtration

methods and the larger inaccuracy introduced by extrapolation. Although additional complexities of interpretation (Gilbert, 1955, 1959) apply to the apparent molecular weight values measured for monomer–oligomer mixtures, values measured under similar circumstances have been thought to relate to weight-average molecular weight and have in any case been adequately validated as indicators of dissociation (Winzor and Scheraga, 1964; Henn and Ackers, 1969). The present experiments provide no information regarding the relative importance or unimportance of dimer and trimer in the dissociation equilibria (see, Klotz *et al.*, 1970) or regarding the catalytic activity of such intermediate species. Another undecided circumstance is the essential identity or nonidentity of monomeric species (*i.e.*, with regard to amino acid sequence, etc.).

As noted, the chromatographic evidence shows association to be enhanced on addition of substrate or the competitive inhibitor *N*-acetylgalactosamine, both of which must therefore be more tightly bound to the more associated species. Since this effect is demonstrable for inhibitor over a range including essentially both extremes of association, it follows that inhibitor and (presumably) substrate are bound most tightly to tetramer and least tightly to monomer. The observed enzymic inactivity of monomer might indeed stem entirely from a limited ability to bind substrate, a circumstance consistent with but not demonstrable from present findings.

To summarize and correlate the physical and kinetic findings, a simple model consistent with both is offered provisionally. Protein–protein binding and protein–ligand binding are considered in this model (Figure 7) to occur at different sites in the same subunit. Such an assumption necessitates a difference in conformation between the monomer and the subunits of the tetramer, to account for observed differences in tightness of ligand binding. The temperature and substrate effects are accounted for by postulating a shift at lower temperatures toward a conformational state (E*) which favors both protein ligand and protein–protein binding. Subunits in the other conformational state postulated (E) must show looser binding of both kinds (or none). Neither the validity of the arguments used in the present qualitative treatment nor the nature of the overall conclusions would appear to be substantially altered by adoption of a more elaborate model, which would consider dimers, trimers, and other species intermediate in the equilibria, or additional conformational states.

The observed small but measurable time lags in change of catalytic activity with change of environment may well refer only indirectly to association and dissociation, potentially processes of extreme rapidity (*e.g.*, Huang and Frieden, 1969), but more directly to concomitant changes in subunit conformation, such as exemplified in the model. As mentioned, both dissociation and the variation of specific activity with enzyme concentration are enhanced at decreased enzyme concentration, at decreased substrate concentration, and at higher temperatures. This behavior is consistent with an allosteric model, since only when the relative proportion of the weakly binding state (monomer) is large should pronounced cooperativity be observed between protein–protein and protein–ligand binding (*cf.* Monod *et al.*, 1965). Preliminary results of a continuing kinetic study show an additional aspect of cooperativity, a sigmoid relationship between velocity and substrate concentration, seen at elevated temperatures (Weissmann and Santiago, unpublished).

Striking effects of small temperature increases in promoting either association or dissociation have been observed with

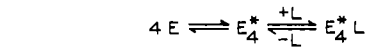

FIGURE 7: A model for kinetic and physical behavior of bovine α-acetylgalactosaminidase. Here, E* represents a monomeric subunit conformation favored at low temperatures and showing preferential ligand–protein binding and protein–protein binding, as compared to an alternative conformational state, E. Substrate or competitive inhibitor is represented by L.

some other proteins (Scrutton and Utter, 1965; Antonini *et al.*, 1966; DeVincenzi and Hedrick, 1970; Constantinides and Deal, 1970) and such effects have been rationalized in at least one case as a temperature-dependent change in subunit conformation (Iwatsuki and Okazaki, 1967). The present relatively simple instance of polymerization allostery is in some ways reminiscent of the reported behavior of arylsulfatase A, whose specific activity also appears to diminish on dilution (Roy, 1953) and whose dissociation also becomes extensive under conditions of assay (Nichol and Roy, 1965). For this enzyme, however, some curious kinetic complexities remain unexplained (see, Roy, 1960) and the effect of substrate on dissociation has apparently not been reported. The low concentrations at which dissociation is demonstrable in the present case and the necessity for supplementing the dilute enzyme with extraneous protein to prevent inactivation have restricted the available range of methodology. Perhaps, similar circumstances account for the sparsely reported occurrence of analogous cases, which may conceivably be common.

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Metabolism of Prostaglandin E_2 in the Rat*

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ABSTRACT: Tritium-labeled prostaglandin E_2 was injected intravenously into female rats in doses ranging from 1.4 mmoles to less than 0.5 nmole per rat. About 55% of the administered radioactivity was excreted into the urine. After extraction with butanol, nine metabolites were isolated and identified.

All metabolites could be isolated after injection of different amounts of prostaglandin E_2 indicating that the identified metabolites can be normally excreted. The identified metabolites are 7α,11-dihydroxy-5-ketotetranorprost-9-enoic acid (tetranorprostaglandin E_1), 11-hydroxy-5-ketotetranorprosta-4(8),9-dienoic acid (tetranorprostaglandin B_1), 11,16-dihydroxy-5-ketotetranorprosta-4(8),9-dienoic acid, 11,15-dihydroxy-5-ketotetranorprosta-4(8),9-dienoic acid, 11-hydroxy-5-ketotetranorprosta-4(8),9-diene-1,16-dioic acid, 5β,7α-dihydroxy-11-ketotetranorprostanoic acid, 7α-hydroxy-5,11-diketotetranorprostanoic acid, 7,16-dihydroxy-5,11-diketotetranorprostanoic acid, and 7-hydroxy-5,11-diketotetranorprosta-1,16-dioic acid. The structures were established by comparison with reference compounds (in some cases), by gas–liquid chromatographic and mass spectrometric analyses of different derivatives of the metabolites, deuterated or not, and by chemical degradations. To study the metabolism of tetranorprostaglandin E_1, tetranorprostaglandin B_1, 5β,7α-dihydroxy-11-ketotetranorprostanoic acid, and 7α-hydroxy-5,11-diketotetranorprostanoic acid, the purified metabolites were injected into rats and the radioactive urinary products were isolated. The results indicate that prostaglandin E_2 probably is degraded to three groups of metabolites *via* three separate pathways.

Early studies of the metabolism of prostaglandin E compounds demonstrated that they are subject to oxidation of the alcohol group at C-15 and to saturation of the Δ^{13} double bond in preparations of lung tissue (Änggård *et al.*, 1965; Änggård and Samuelsson, 1964, 1965). The dehydrogenase, catalyzing the oxidation at C-15, has been purified and it was found to be active on a large number of different prostaglandins (Änggård and Samuelsson, 1966). It has also been shown that prostaglandins incubated with rat liver mitochondria undergo one or two steps of β oxidation forming dinor and tetranor compounds, respectively (Hamberg, 1968). Recently the formation of 19- and 20-hydroxylated derivatives from prostaglandin A_1 and B_1 in incubations with guinea pig liver microsomes has been demonstrated (Israelsson *et al.*, 1969).

The major urinary metabolite of PGE_2[1] in man and guinea pig has been identified as 7α-hydroxy-5,11-diketotetranorprosta-1,16-dioic acid and 5β,7α-dihydroxy-11-ketotetranorprostanoic acid, respectively (Hamberg and Samuelsson, 1969a,b). The human metabolite is formed from PGE_2 after four types of reactions have taken place: (1) oxidation of the alcohol group at C-15 (Änggård *et al.*, 1965), (2) reduction of the trans double bond (Änggård *et al.*, 1965), (3) two steps

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[1] The abbreviations used are: PGE_1, prostaglandin E_1, 11α,15-dihydroxy-9-ketoprost-13-*trans*-enoic acid; PGE_2, prostaglandin E_2, 11α,15-dihydroxy-9-ketoprosta-5-*cis*,13*trans*-dienoic acid; PGE_3, prostaglandin E_3, 11α,15-dihydroxy-9-ketoprosta-5-*cis*,13-*trans*,17-*cis*-trienoic acid; PGA_1, prostaglandin A_1, 15-hydroxy-9-ketoprosta-10,13-dienoic acid; PGB_1, prostaglandin B_1, 15-hydroxy-9-ketoprosta-8(12),13-dienoic acid; $PGF_1\alpha$, prostaglandin $F_1\alpha$, 9α,11α,15-trihydroxyprost-13-*trans*-enoic acid; $PGF_2\alpha$, prostaglandin $F_2\alpha$, 9α,11α,15-trihydroxyprosta-5-*cis*,13-*trans*-dienoic acid; MO, *O*-methyloxime; TMSi, trimethylsilyl ether.