Ces expériences montrent que la fluorophénylalanine peut s'incorporer dans l'hémoglobine en se substituant partiellement à la phénylalanine.

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Preliminary Notes

Formation of L-gulonolactone from D-glucuronolactone with TPN L-gulonic dehydrogenase

It has been established by several workers that the immediate precursor of L-ascorbic acid in animal tissue is L-gulonolactone[1-4]. However, the mechanism of formation of the lactone has not yet been elucidated completely. Recently, YAMADA[5] demonstrated the enzymic formation of L-gulonolactone from L-gulonate by the reverse reaction of lactonase I, but HERS[6] suggested the direct conversion of D-glucuronolactone to L-gulonolactone without any consideration for the action of lactonase. The present paper describes the enzymic formation of L-gulonolactone from D-glucuronolactone by the action of TPN gulonic dehydrogenase which was considered to operate with D-glucuronate[7].

The supernatant fraction[8] from rat liver was subjected to $(NH_4)_2SO_4$ fractionation (0.40–0.50 satn.), dialysis, and fractionation on DEAE-cellulose column. The second

Abbreviations: TPN, TPNH, oxidized and reduced triphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)-aminomethane.

fraction eluted with 0.02 *M* Tris, pH 7.5, usually showed a specific activity 350–500 times that of the original extract. The enzyme catalyzed the reduction of D-glucuronolactone as well as D-glucuronate by TPNH in spite of the complete absence of the lactonase. DPNH could not substitute for TPNH. Spontaneous degradation of the lactone to its acid could be neglected under the conditions tested. Thus, the direct conversion of D-glucuronolactone to L-gulonolactone appeared reasonable, and this was supported also by the following evidence: (1) the initial velocity of reaction of the lactone was greater than that of the acid in concns. of over $1.54 \cdot 10^{-3} M$; (2) the ratio of the activity of the lactone to that of the acid was constantly 1.2 over each step of the purification; (3) the ratio of activity to two substrates was in good agreement in each effluent of the chromatography, (4) various animal organs lacking lactonase I activity[3] were found to be active with D-glucuronolactone[3]; (5) the latter was active as substrate of the enzyme in the presence of *p*-chloromercuribenzoate which inhibits the lactonase completely[9,10]; and (6) the reaction product from D-glucuronolactone was identified as L-gulonolactone by paper chromatography with three solvent systems, by periodate consumption and by hydroxamate formation[9]. The enzyme also catalyzed the reduction of other derivatives of various sugars* (Table I). Lower aldomonosaccharides could also serve as a substrate, but higher sugars, both mono- or polysaccharides, were inactive. This pattern of specificity closely resembles that of aldose reductase[6] except for the inactivity of higher sugars. The reductions of D-glucuronolactone and D-glucosone were not reversible.

The results show that L-gulonolactone may be formed from D-glucuronolactone directly by coupling with TPNH, and if the latter is present in animal tissue the formation of L-gulonolactone by direct conversion may be more favorable than the secondary lactonization[4,5]. The effectiveness of D-galacturonic ester as substrate suggests the formation of D-galactonolactone if its mechanism is the same as in plant system[11]. Furthermore, gulonolactone dehydrogenase[12], the enzyme which catalyzes the oxidation of L-gulonolactone to L-ascorbic acid, and lactonase I[9,10] act on deriva-

TABLE I

SPECIFICITY OF THE ENZYME

The reaction mixture contained phosphate buffer, 50 μmoles; TPN or TPNH, 0.5 μmole; substrate, 10 μmoles; enzyme, 30 μg. Total vol., 3.0 ml; pH, 7.2. Incubation for 10 min at 37°. The change of absorbancy at 340 mμ was determined.

Substrate	*Activity of reduction*	*Substrate*	*Activity of dehydrogenation*
D-Glucuronate	0.154	L-Gulonate	0.033
D-Glucurono-γ-lactone	0.182	L-Gulono-γ-lactone	0.003
Ethyl D-glucuronate	0.145	L-Galactonate	0.120
D-Galacturonate	0.194	L-Galactono-γ-lactone	0.068
Methyl D-galacturonate	0.162	D-Galactonate	0
D-Mannuronate	0.180	D-Galactono-γ-lactone	0
D-Mannurono-γ-lactone	0.192	D-Fructose	0.004
D-Glucosone	0.178	Glycerol	0.038
DL-Glyceraldehyde	0.227		
Glycolaldehyde	0.052		

* This preparation was free from enzymes which catalyze uronic acid interconversion[14,15].

tives of galactose more easily than on those of glucose. It seems probable, therefore, that the mechanism of L-ascorbic acid biosynthesis in animal tissue is in substantial agreement with that found with plant system[13].

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Isolement et composition en acides aminés d'une substance glycopeptidique formée par action de la présure sur la caséine du lait de vache

La caséine du lait de vache, soumise à l'action de la présure ("rennin"), subit à pH 6.8 une protéolyse rapide mais limitée; il apparaît dans le filtrat trichloracétique des substances azotées solubles, qui représentent de 1 à 3.5 % de l'azote de la caséine, selon les conditions de déprotéinisation et, en particulier, selon la concentration en acide trichloracétique[1]. Des études préliminaires[2,3] ont montré que la substance soluble dans l'acide trichloracétique à 12 % de concentration finale ("NPN-12 %") est de nature glycopeptidique, ne dialyse pas à travers les membranes de cellulose, contient moins de phosphore que la caséine de départ, aucun acide aminé aromatique et renferme une partie non peptidique, dans laquelle on a trouvé de la glucosamine, du galactose et de l'acide neuraminique.

Le "NPN-12 %" provenant de la caséine entière et le "NPN-12 %" provenant de la caséine α de lait de vache, après dialyse poussée, contiennent 13 % d'azote. Chacune de ces substances a été soumise à des essais de purification par passage sur colonne de résine échangeur d'ions.

Le "NPN-12 %" de caséine α par passage sur Dowex 50-X2 donne naissance à trois fractions principales A_α, B_α et C_α, représentant respectivement 41 %, 14 % et 43 % du produit de départ (Fig. 1).

Le "NPN-12 %" de caséine entière se comporte d'une façon analogue et la chromatographie permet également de repérer trois fractions principales: A, B et C (40 %, 10 %, 50 %).