The kinetic parameters of the fragments in inhibition of tryptic activities[a]

	I_{50}[b] (μM)		K_i[a] (μM)	
	Tos-Arg-OMe	Gly_2-Lys-Gly_3	Tos-Arg-OMe	Gly_2-Lys-Gly_3
Ia	40	3.6	15	1.5
Ib	330	13	99	3.8

[a]The kinetic measurement was carried out under the conditions described in Figure 2: the substrate concentration was selected in the range of 0.33–1.0 mM for esterase activity, and 1.0–3.0 mM for peptidase activity. [b]The substrate concentration was 1 mM and 3 mM for Tos-Arg-OMe and Gly_2-Lys-Gly_3, respectively.

Results and discussion. The gel filtration of crude Ia on Sephadex G-25 gave 3 peaks. Materials obtained from peak 2 and 3 were reduced separately with β-mercaptoethanol at pH 8.6 and applied to the column giving a main peak at the same position to peak 3; Rf (Gel) 0.61 (Figure 1A), positive by nitroprusside. The similar results were obtained for the crude cyclic nonapeptide Ib (Figure 1B). On paper electrophoregrams the peak 2 moved to cathode, R 0.49 × Lys, and peak 3, R 0.44 × Lys. Reduced peak 2 or 3 showed the same mobility as peak 3 itself. Similar results were obtained for the materials related to Ib. These observations suggest that the peak 3 and 3′ are monomers and peak 2 and 2′ are presumed to be dimers.

Figure 2 shows that both of Ia and Ib inhibit esterase and peptidase activities of trypsin. However, these small fragments did not form stable 1:1 complex with trypsin. The value of I_{50}[15], the concentration of an inhibitor in which tryptic activity was suppressed to 50%, was given in the Table for each fragment.

The results of kinetic measurements indicate that the mode of inhibition was competitive. The K_i values of fragment Ia and Ib were determined by Dixon's plot and shown in the Table. The difference of K_i values of fragments between esterase and peptidase activity seems to be attributable to that of the K_m value of each substrate. After Ia (10 mg) was treated with trypsin at pH 3.75 as described in the literature[16], the digested fragment (Ia*) which had been hydrolized at Lys-Ser (16–17) bond was isolated by gel filtration as described above (Rf (Gel) 0.69) and ascertained by paper electrophoresis (R 0.63 × Lys). Ia* did not exhibit any inhibitory activity. The fragment Ia did not inhibit chymotryptic activity when Gly_2-Tyr-Gly_3[17] was used as a substrate. Another nonapeptide (41–49) containing antichymotryptic site and its analogs are under synthesis in this laboratory.

Zusammenfassung. Zwei Nonapeptidfragmente des Bowman-Birk Inhibitors wurden mit Hilfe der Merrifield-Synthese hergestellt und deren Trypsinhemmende Aktivität bestimmt.

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The Association of Enzymic Activities in Subfractions of Isolated Rat Liver Golgi

The Golgi apparatus has been implicated in the biosynthesis of glycoproteins and lipoproteins and in sulphate metabolism[1–3]. It is tempting to speculate that the different enzymic activities are localized to discrete areas of this complex organelle. In our experiments it has been possible to show some separation of activities after mild homogenization and centrifugation in sucrose gradients.

Materials and methods. The Golgi fraction was isolated from male Wistar rats (200–220 g), fasted 16–18 h, by the procedure described previously[4]. Golgi fractions from 2–3 livers were collected in a volume of not more than 2 ml and were homogenized at 80 rpm with 3 up and down passes with a tygon pestle having a clearance of 0.006 inches. This homogenate was placed on a sucrose gradient of densities 1.20, 1.18, 1.16 and 1.14, containing 5 mM $MgCl_2$, 3 mM mercaptoethanol and 37.5 mM *Tris*-maleate buffer, pH 6.4. The gradients were centrifuged at 30,000 rpm for 3 h in SW-50.1 Beckman rotor. 3 bands were isolated and kept frozen at −18 °C until enzymes were assayed. About 25–30% of the original Golgi protein was recovered with a distribution of 66 in the first, 23 in the second, and 11% in the third band. The enzymes assayed have been chosen for their reported presence or suggested involvement in the activities in the Golgi-GERL[5] system[1–3], as well as non-Golgi enzymes to monitor purity of the preparations that were assayed.

Enzyme assays. The following enzymes were assayed. Acid phosphatase (EC: 3.1.3.2)[6]; alkaline phosphatase (3.1.3.1)[7]; arylsulfatase-A (3.1.6.1) and arylsulfatase-B (3.1.6.1)[8,9]; thiamine pyrophosphatase[10]; galactosyl-

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Table I. The specific activities of several golgi and non-golgi enzymes in homogenates of rat liver, isolated golgi and subfractions of isolated golgi

Enzymes assayed	Whole liver homogenate	Golgi fraction	Band I	Band II	Band III
Acid Phosphatase[a]	0.47	0.45	0.65	0.05	ND
	0.37–0.52	0.28–0.68	0.30–0.86	0–0.15	
Alkaline Phosphatase[a]	0.97	1.46	2.46	8.30	3.33
	0.68–1.42	0.92–2.10	0.48–4.96	4.50–15.20	1.0–6.2
Thiamine	10.90	19.2	20.0	69.3	3.3
Pyro-Phosphatase[b]	8.8–13.7	14.9–25.0	13.4–30.0	37.4–116.2	0.5–13.0
Galactosyl Transferase[c]	3.4	120.1	198.4	29.1	14.1
	2.1–6.0	68.6–208	160–246	9.4–67.4	1.2–30.5
Aryl-Sulfatase A[d]	16.4	14.4	8.5	12.1	39.7
	12.6–21.4	9.2–18.6	6.8–12.6	6.7–20.0	38.8–70.0
Aryl-Sulfatase B[d]	37.0	20.0	9.7	33.8	ND
	27.0–67.0	14.7–28.0	6.0–19.2	18.2–72.8	
Glucose-6-Phosphatase[e]	0.21	0.03	0.01	0.01	ND
	0.17–0.27	0.01–0.04	0–0.03	0–0.02	
5′-Nucleotidase[e]	0.05	0.01	<0.01	Traces	ND
	0.03–0.08	0.01–0.02	0–0.02		
Malic-dehydrogenase[f]	4.28	3.38	1.76	1.18	ND
	2.8–6.8	1.7–5.2	0.82–3.10	0–2.20	
Malic Enzymes[g]	0.14	0.20	0.01	ND	ND
	0.12–0.16	0.13–0.33	0–0.04		
Urate Oxidase[h]	0.58	0.02	Traces	ND	ND
	0.36–0.77	0–0.05			

[a] Acid and alkaline phosphatases (μM *p*-nitrophenol liberated/min/mg/protein). [b] nM inorganic phosphorous liberated/min/mg protein. [c] nmol of galactose transferred/h/mg protein .[d] nM *p*-nitrocatechol liberated/min/mg protein. [e] μM inorganic phosphorous liberated/min/mg protein. [f] nM NAD reduced/min/mg protein. [g] Ochoa units. [h] μM uric acid destroyed/min/mg protein. ND, not detected.

transferase[11]; urate oxidase (1.7.3.3.)[12]; glucose-6-phosphatase (3.1.3.9)[13]; NAD-malic dehydrogenase (1.1.1.37)[14]; 'malic-enzymes' (1.1.1.38, 1.1.1.39, 1.1.1.40)[15]; 5′nucleotidase[16] and total protein[17].

Results and discussions. Centrifugation of the Golgi homogenate resulted in separation of three bands with densities of 1.15, 1.17 and 1.19. The specific activities of enzymes assayed in each band are compared to those for the liver homogenate and the isolated Golgi fractions in Table I. The data in Table I support the view that some separation of Golgi enzymes was achieved. In Table II the percentage of total activity recovered in each band as compared to that of the Golgi homogenate applied to the final gradient is shown.

The galactosyltransferase was almost exclusively localized to Band I, which contained large masses of tubules when examined by electron microscopy after negative staining. Alkaline phosphatase, thiamine pyrophosphatase and probably arylsulphatase B are associated in Band II which contains small membrane fragments and vesicles. Arylsulphatase A was found in Band III which consisted of large membrane fragments but contained only 19% of activity of that in the Golgi homogenate. Band I contained 16% of the activity, so that it was difficult to decide on the basis of total enzyme activity whether or not some localization of this activity had occurred. The separation of enzyme activities in membranes sedimenting at different densities in the sucrose gradient

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Table II. Recovery of enzyme activities in golgi subfractions (% activity[a])

Sample	Gal transferase	Acid phosphatase	Alkaline phosphatase[b]	Thiamine pyrophosphatase	Aryl sulfatase A	Aryl sulfatase B
Band I	47	39	41	28	16	13
Band II	3	3	74	48	11	24
Band III	0.7	Not detected	16	1.3	19	Not detected

[a] $\%\ \text{activity} = \dfrac{\text{Total activity in Band} \times 100}{\text{Total activity in Golgi homogenate}}$

[b] The recoveries of alkaline phosphatase were always anomalous and varied between 120–150% of that of the Golgi homogenate. Total activities; specific activity × total protein.

implies that different membranes possess unique enzyme activities which must reflect the functional activity of particular membrane systems.

Because of the vastly different experimental procedures used, it is difficult to compare the present study with those of OVTRACHT et al.[18] or BERGERON et al.[19]. In the work of OVTRACHT et al.[18], a crude enzyme mixture, takadiastase, was used which contains many different activities including proteases, glycosidases, phosphatases, RNases, etc.[20], it is difficult to separate enzyme activities which may be a part of the Golgi system as compared to those which may have become associated with the membranes after addition of takadiastase. A similar objection can be made to the use of lysosomal extracts to unstack Golgi. The use of large doses of ethanol by BERGERON et al.[20] results in toxic effects, which would be reflected in fluctuations of the enzymic activities. A recent report describes a nearly 100% increase of glycosyltransferase activities in rat liver Golgi after a single dose of ethanol[21]. An increase of ^{35}S incorporation into liver mucopolysaccharides after alcohol has also been reported[22]. We feel that the gentle homogenization procedure used in our laboratory represents a fair assessment of associated enzyme activities[23]

Zusammenfassung. Aus Rattenleber isolierte Golgi Apparate wurden unter milden Bedingungen homogenisiert und auf diskontinuierlichen Sucrose-Gradienten aufgetragen. Beim Zentrifugieren wurden 3 Banden mit jeweils Anreicherungen mehrerer Enzyme erhalten, welche einer partiellen Verteilung der Enzymaktivitäten entsprechen.

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Inhibition of Liver Aldehyde Dehydrogenase by Pyrogallol and Related Compounds

The observation that in vivo blood levels of acetaldehyde (AcH), the initial and probably most reactive intermediate in the metabolism of ethanol (EtOH), are significantly elevated during alcohol intoxication in rats if pyrogallol (PG; 1,2,3-trihydroxybenzene) is pre-administered[1,2] has prompted us to examine the effect of PG and several related derivatives on liver aldehyde dehydrogenase (AldDH; EC 1.2.1.3). It has been previously shown[2] that the increase in blood AcH levels in vivo following PG and EtOH is not contingent upon PG's known inhibition of catechol-*O*-methyltransferase (COMT) and probably is not mediated by activation of catalase-dependent EtOH metabolism. The effect of PG, PG metabolites, or related catechol compounds on the activity of AldDH has not been investigated.

Materials and methods. All chemicals were of the highest commercial quality. Mitochondrial AldDH was prepared from livers of male Sprague-Dawley rats essentially as described by TABAKOFF et al.[3], except that the disruption of isolated mitochondria was accomplished by 4 one-min sonication periods using a Bramson Sonifier at a power setting of 4. The final solution of AldDH was shown to be essentially free of monoamine oxidase (MAO) activity. MAO activity was assayed as described by TABAKOFF and ALIVISATOS[4] and was found to be $< 5\%$ of the activity found in the mitochondrial pellet.

Table I. Effect of pyrogallol and related compounds on in vitro activity of rat liver mitochondrial aldehyde dehydrogenase

Compound	Inhibition (%) Concentration (m*M*) 0.05	0.5	1.0
PG (1,2,3-trihydroxybenzene)	6.4	27.0	49.5
1,2,4-Trihydroxybenzene	38.5	49.5	60.0
Hydroquinone (1,4-dihydroxybenzene)	26.2	41.9	54.1
Chloral hydrate	—	—	56.2
DDC (diethyldithiocarbamate)	6.8	26.7	33.3
3-Methoxy-*O*-catechol	0	17.2	31.4
2,3-Dimethoxyphenol	0	4.3	6.9
Gallic acid	0	0	15.6
n-Propyl gallate	0	0	6.2
D,L-Shikimic acid	0	0	3.4
Tetrahydroxy-*p*-quinone	0	0	0

Aldehyde dehydrogenase activity was assayed at 25 °C in incubation mixtures containing 0.05 *M* Na_3PO_4, pH 7.0, 1 m*M* NAD^+, and enzyme (1–2 mg protein). The reactions were initiated by the addition of substrate, propionaldehyde, (2 m*M*). Measurements of AldDH activity were performed at pH 7, due to rapid oxidation of PG at higher pH's. For kinetic studies, the concentration of either NAD^+ or propionaldehyde was varied while the other components of the incubation mixture were kept constant. Inhibitors, when present, were preincubated with enzyme for 2 min before the addition of substrate. Incubations without inhibitor were assayed simultaneously with each incubation containing inhibitor. Formation of NADH was monitored spectrophotometrically using a Beckman Acta III recording spectrophotometer with a 0.1 absorbance unit, full scale expansion. Kinetic data, plotted as described by LINEWEAVER and BURK[5], were fitted by linear regression analysis using an Olivetti Programma 101. Each point represents the mean of 3 determinations, S.D. $< 7\%$.

To ascertain whether the inhibition by PG was reversible, AldDH was pre-incubated with PG (10 m*M*) for

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