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Calcium transport by basal lateral membrane vesicles from rat small intestine decreases with age

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There is a marked decrease in active Ca^{2+} transport by the rat small intestine with age, particularly between 2 and 12 months. Much evidence suggests that the active component of Ca^{2+} transport resides in the energy-dependent pumping of Ca^{2+} across the intestinal basal lateral membrane. Therefore, we have characterized Ca^{2+} uptake by basal lateral membrane vesicles isolated from young (2–3 month old) and adult (12–14 month old) rats. In vesicles from the proximal duodenum, ATP-dependent Ca^{2+} uptake was about 4-times greater in the young animal than in the adult. There were no age differences in Ca^{2+} uptake in the absence of ATP. In vesicles from the ileum, Ca^{2+} uptake was much less than in the duodenum. The age differences in the ileum were smaller, and ATP-dependent Ca^{2+} uptake in the young was only twice that seen in the adult. Osmotic lysis of duodenal vesicles reduced Ca^{2+} uptake to low levels in both age groups, indicating that most of the Ca^{2+} was being taken up into an osmotically active space. Kinetic studies of Ca^{2+} uptake showed that there was no change in the apparent affinity but a 5-fold decrease in the V_{max} of the adult Ca^{2+} transport system compared to that of the young animal. This marked decrease in the capacity of basal lateral membrane vesicles to actively transport Ca^{2+} may contribute to the decline in intestinal Ca^{2+} absorption with age.

Introduction

The rate of absorption of Ca^{2+} by the intestine declines with age in both humans [1] and rats [2,3]. Studies in rats have suggested that it is the active, energy-dependent component of Ca^{2+} absorption which decreases with age. Everted intestinal sacs from the duodenum of young animals actively transport Ca^{2+}, while those from adult animals do not [2]. In terms of intestinal uptake of Ca^{2+}, the greatest age-related decrease is seen in the proximal duodenum [3], which is the site of energy-dependent Ca^{2+} transport. The ileum, which demonstrates little energy-dependent Ca^{2+} transport, shows no change in Ca^{2+} uptake with age [3].

Much evidence suggests that the basal lateral membrane of the intestinal absorptive cell is the site of the energy-dependent component of Ca^{2+} transport. Basal lateral membrane vesicles (BLMV) accumulate Ca^{2+} in the presence of ATP [4–6], and they contain Ca^{2+}-ATPase activity [7]. Ca^{2+} uptake by BLMV is stimulated by 1,25-dihydroxyvitamin D [8], the hormonal form of vitamin D

Abbreviations: BLMV, basal lateral membrane vesicles; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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which increases intestinal Ca^{2+} absorption. In addition, BLMV isolated from the duodenum take up much more Ca^{2+} than membranes isolated from the ileum [8].

The purpose of the studies reported here was to determine whether the capacity of intestinal BLMV to actively accumulate Ca^{2+} changes with age. Ca^{2+} accumulation was compared between rats of 2 and 12 months of age, since the greatest decline in Ca^{2+} uptake and active transport takes place during that age span [2,3]. Age-related changes in the duodenum were also compared to those in the ileum. Finally, changes in Ca^{2+} accumulation were characterized kinetically to determine whether there were changes in the number and/or affinity of the Ca^{2+} transporters with age.

Materials and Methods

Experiments were performed using male Fischer 344 rats purchased from Harlan Industries (Indianapolis, IN). Rats were 2–3 months (young) or 12–14 months (adult) of age. Animals were maintained on Purina laboratory rodent chow (Ralston Purina Co., St. Louis, MO) containing 1.2% Ca, 0.8% P and 3.3 IU/g vitamin D.

BLMV were isolated from intestinal segments by differential and density gradient centrifugation [7]. Intestinal segments from three animals were pooled for a single preparation. Duodenal BLMV were isolated from tissue 0–10 cm distal to the pylorus, and ileal BLMV were isolated from tissue 0–10 cm proximal to the caecum. Intestinal cells were isolated by filling 5-cm intestinal sacs with a citrate solution (1.5 mM KCl/96 mM NaCl/27 mM sodium citrate/8 mM potassium phosphate/5.6 mM sodium biphosphate, pH 7.3) [9]. After a 15 min incubation at 37°C, epithelial cells were released by gently pressing on the outside of the sac [9]. The cells, consisting primarily of columnar epithelial cells with a few crypt cells [9], were collected by low-speed centrifugation and resuspended in a low ionic strength buffer (25 mM NaCl/1 mM Tris-Hepes, pH 8.0/0.2 mM phenylmethanesulphonyl fluoride).

To isolate the BLMV, the cells were disrupted by using a Polytron homogenizer for 1 min [7]. The homogenate was centrifuged ($550 \times g$, 15 min) to remove brush borders and cell debris. The supernatant was then centrifuged ($90\,000 \times g$, 20 min). The resulting pellet was resuspended in sorbitol buffer (250 mM sorbitol/12.5 mM NaCl/0.5 mM EDTA/5 mM Hepes-Tris, pH 7.4) by homogenization with a glass/teflon homogenizer. Basal lateral membranes were separated from mitochondria by density gradient centrifugation. The sorbitol concentration of the resuspended pellet was increased to 40% (w/w) and this was overlaid with 25% sorbitol in a centrifuge tube. This was centrifuged ($95\,000 \times g$, 60 min), and the material at the 25/40% interface, containing purified basal lateral membranes, was collected. Basal lateral membranes were diluted 6-fold with 200 mM sorbitol/5 mM Tris-Hepes (pH 7.4) and collected by centrifugation ($100\,000 \times g$, 15 min). The resulting pellet was resuspended in uptake buffer (100 mM KCl/5 mM $MgCl_2$/20 mM Tris-Hepes, pH 7.4).

The purification and recovery of the BLMV was monitored using Na^+/K^+-ATPase as a marker [10]. Membrane protein was measured by the method of Lowry [11]. There was no difference between young and adult animals in the purification and recovery of this marker. In five separate BLMV preparations, the purification factor was 16.1 ± 3.8 and 15.8 ± 2.5 for young and adult animals, respectively. The recovery was $45.5 \pm 5.8\%$ for the young and $42.5 \pm 7.1\%$ for the adult. This compares favorably with a purification factor of 10.3 and a recovery of 15.4% previously reported for this isolation procedure [7]. In addition, this BLMV preparation shows no sodium-dependent glucose uptake such as is seen in brush-border membrane vesicles [12]. This suggests that the preparation is relatively free of contamination by brush-border membrane vesicles.

Ca^{2+} uptake by BLMV was measured by filtration [7]. Uptake was initiated by adding 50 μl of a radiolabeled Ca^{2+} solution to 50 μl of BLMV (50–150 μg protein). The Ca^{2+} solution usually consisted of uptake buffer containing 10 μM calcym chloride and labeled with $^{45}Ca^{2+}$. This gave a final Ca^{2+} concentration of 5 μM. When present, the final Tris-ATP concentration was 5 mM. In the kinetic experiments (Figs. 2 and 3), the Ca^{2+} concentration was varied between 0.02 and 0.9 μM by using Ca^{2+}-EGTA buffers [13]. After incubation at 25°C for the required time,

the reaction was terminated by the addition of 1 ml of cold stop solution (100 mM KCl/5 mM $MgCl_2$/1 mM $LaCl_3$/20 mM Tris-Hepes, pH 7.4). The mixture was then filtered through a 0.45-μm Millipore filter (HAWP 02500), and the filter was washed twice with 2 ml of stop solution. Radioactivity on the filters was quantitated by liquid scintillation spectrometry using ACS scintillation fluid (Amersham, Arlington Heights, IL). Nonspecific binding to the filters, assayed in the absence of membrane protein, was typically less than 5% of Ca^{2+} uptake by young BLMV in the presence of ATP.

Results are presented as the mean ± S.E. of triplicate determinations using the same membrane preparation. In most cases, the results of individual experiments are presented. However, all experiments shown were performed at least twice with similar results. The absolute magnitude of Ca^{2+} uptake varied from preparation to preparation, but the pattern of Ca^{2+} uptake in each experiment was very reproduceable. Statistical analysis was performed using a two-tailed Student's *t*-test [14], and a confidence level of 95% or greater was considered significant.

Results

Ca^{2+} uptake by BLMV isolated from the proximal duodenum of young and adult rats was measured as a function of time (Fig. 1). In general, Ca^{2+} uptake was rapid during the first 5 min and then plateaued. In the young rats, ATP markedly stimulated Ca^{2+} uptake at all time points. In contrast, ATP increased Ca^{2+} uptake only modestly in the adult rats. After 10 min, Ca^{2+} uptake in the presence of ATP was more than four times greater in the young rat compared to that in the adult.

Ca^{2+} uptake was compared as a function of the intestinal segment in young and adult rats (Table I). In the duodenum, there was no age difference in Ca^{2+} uptake in the absence of ATP. In the presence of ATP, Ca^{2+} uptake was much greater in the young as compared to that in the adult rat, as can be seen in Fig. 1. ATP-dependent Ca^{2+} uptake, the difference between uptake in the presence and absence of ATP, was about four times greater in the young than the adult.

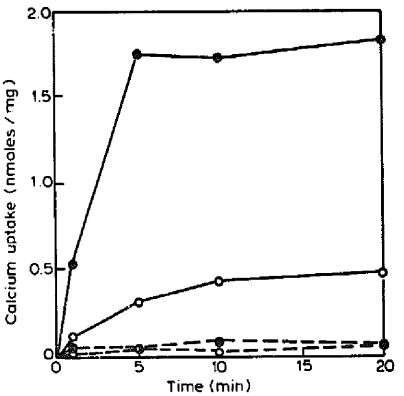

Fig. 1. Time course of Ca^{2+} uptake by basal lateral membrane vesicles. Ca^{2+} uptake was measured at the indicated time by filtration. Uptake was measured at a Ca^{2+} concentration of 5 μM Ca in the presence or absence of 5 mM ATP. Data points are the mean of triplicate determinations and S.E. was less than the size of the symbol. ●———●, young rat (+ATP); ○———○, adult rat (+ATP); ●------●, young rat (−ATP); ○------○, adult rat (−ATP).

In the ileum, differences in Ca^{2+} uptake between young and adult rats were seen, but the differences were much smaller than those in the duodenum (Table I). ATP modestly stimulated Ca^{2+} uptake in BLMV from both the young and adult rats, but ATP-dependent Ca^{2+} uptake in the young animals was only twice that found in the

TABLE I

EFFECT OF INTESTINAL SEGMENT ON Ca^{2+} UPTAKE BY BASAL LATERAL MEMBRANE VESICLES

Values are the mean ± S.E. of triplicate determinations. Ca^{2+} uptake was measured by filtration after 10 min in the presence of 5 μM Ca^{2+}. ATP-dependent Ca^{2+} uptake represents the difference between Ca^{2+} uptake in the presence and absence of 5 mM ATP.

Age	Segment	Ca^{2+} uptake (nmol/mg)		
		−ATP	+ATP	ATP-dependent
Young	duodenum	0.07 ± 0.01	1.71 ± 0.15	1.64 ± 0.16
Adult	duodenum	0.05 ± 0.01	0.42 ± 0.04 *	0.37 ± 0.05 *
Young	ileum	0.09 ± 0.01	0.38 ± 0.01	0.29 ± 0.02
Adult	ileum	0.07 ± 0.01	0.22 ± 0.02 *	0.15 ± 0.02 *

* Significantly different from young animals ($P < 0.05$, Student's *t*-test).

adult. In the absence of ATP, Ca^{2+} uptake in the ileum was the same in young and in adult rats.

To characterize the nature of Ca^{2+} accumulation by BLMV, uptake was measured in the presence of ATP and various inhibitors (Table II). Orthovanadate inhibited Ca^{2+} uptake by 75% in young animals but only by 13% in adult animals. This suggested that the young animals had a much larger component of ATP-dependent Ca^{2+} uptake, since orthovanadate inhibits ATP-dependent Ca^{2+} transport and Ca^{2+}-ATPase activity in BLMV [7]. Oligomycin, an inhibitor of mitochondrial Ca^{2+} uptake, reduced Ca^{2+} uptake only slightly (11–14%) in each age group. This excluded mitochondrial contamination of the BLMV preparation as a major contributor to the ATP-dependent Ca^{2+} uptake. Finally, lysis of the BLMV by distilled water after Ca^{2+} accumulation reduced uptake to low levels in both age groups. This indicated that almost all of the Ca^{2+} was being taken up into an osmotically active space rather than being bound to the vesicle membrane.

To characterize further the differences in Ca^{2+} transport between young and adult rats, we performed kinetic studies of Ca^{2+} uptake by BLMV. Since Ca^{2+} uptake is linear with time during the first 2 min [7], we measured the initial velocity of Ca^{2+} uptake (V) during those first 2 min. Initial velocity was measured at Ca^{2+} concentrations ranging from 0.02 to 0.9 μM (Fig. 2). At each Ca^{2+} concentration, the initial velocity was much greater in BLMV from young rats compared to adult rats.

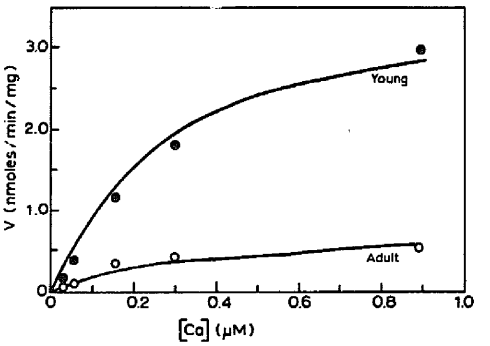

Fig. 2. Effect of Ca^{2+} concentration on ATP-dependent Ca^{2+} uptake by basal lateral membrane vesicles. Ca^{2+} uptake during the first 2 min, V, was measured at the indicated Ca^{2+} concentration in the presence and absence of 5 mM ATP. ATP-dependent Ca^{2+} uptake was calculated by subtracting uptake in the absence of ATP from uptake in the presence of ATP at each concentration of Ca^{2+}. Data points are the mean of triplicate determinations.

To evaluate these data kinetically, the data were replotted using an Eadie-Hofstee plot (Fig. 3). When the data were plotted in this way, the data points fell in a straight line for both young and adult animals. This indicated that the initial

TABLE II

EFFECT OF INHIBITORS ON Ca^{2+} UPTAKE BY BASAL LATERAL MEMBRANE VESICLES FROM THE DUODENUM

Values are the mean ± S.E. of triplicate determinations. Ca^{2+} uptake was measured by filtration after 10 min in the presence of 5 μM Ca^{2+} and 5 mM ATP. The final concentration of sodium orthovanadate was 0.1 mM and of oligomycin was 10 μg/ml. Osmotic lysis was performed by terminating Ca^{2+} uptake with distilled water rather than with the saline stop solution.

Condition	Ca^{2+} uptake (nmol/mg)	
	Young	Adult
Control	2.26 ± 0.28	0.45 ± 0.01
Orthovanadate	0.58 ± 0.03 *	0.39 ± 0.01 *
Oligomycin	2.02 ± 0.13	0.38 ± 0.02 *
Osmotic lysis	0.18 ± 0.01 *	0.23 ± 0.01 *

* Significantly different from control ($P < 0.05$, Student's t-test).

TABLE III

EFFECT OF AGE ON INTESTINAL Ca ABSORPTION

Values are the mean ± S.E. of triplicate determinations (BLMV uptake), six animals (tissue uptake) or four to eight animals (tissue transport). Values for BLMV uptake are from Table I, values for tissue uptake are from Ref. 3 and values for tissue transport are from Ref. 2.

Age	Segment	BLMV uptake (nmol/mg)	Tissue uptake (pmol/mg)	Tissue active transport (S/M)
Young	duodenum	1.64 ± 0.16	205 ± 16	2.7 ± 0.4
Adult	duodenum	0.37 ± 0.05 *	110 ± 13 *	0.7 ± 0.1 *
Young	ileum	0.29 ± 0.02	94 ± 7	–
Adult	ileum	0.15 ± 0.02 *	115 ± 20	–

* Significantly different from values for young rats ($P < 0.05$, Student's t-test).

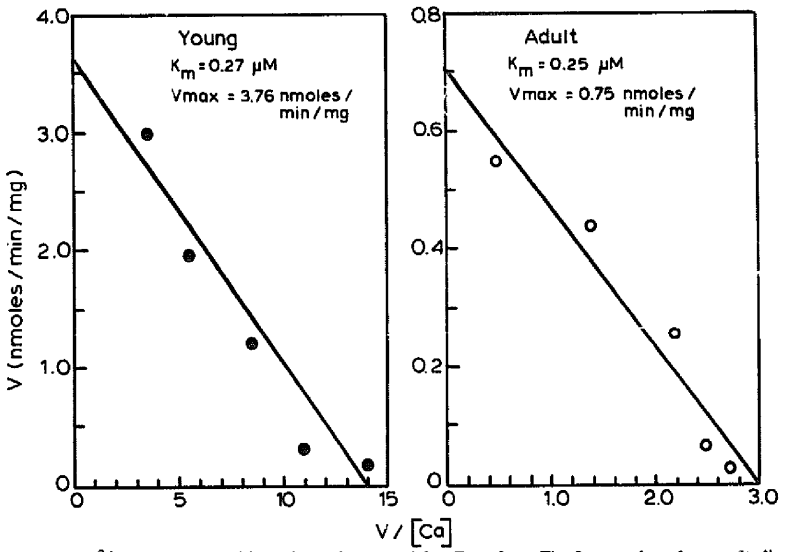

Fig. 3. Kinetic studies of Ca^{2+} uptake by basal lateral membrane vesicles. Data from Fig. 2 were plotted on an Eadie-Hofstee plot to determine the K_m and V_{max} values graphically. Data points were fitted by linear least-squares lines.

velocity of Ca^{2+} uptake followed Michaelis-Menten kinetics to a first approximation. In comparing the kinetic data, the slope of the curves for the two age groups were almost identical. For the young animals, the slope, which is the apparent affinity of the transport system, was 0.27 μM. For the adults, the apparent affinity was 0.25 μM, almost identical to that of the young animals. However, there was a large difference in the *y*-intercepts of the curves. For the young rats, the *y*-intercept, which is the maximal velocity of the transport system, was 3.76 nmol/min per mg. For the adults, the maximal velocity was 0.75 nmol/min per mg. The kinetic parameters of the young BLMV, 0.27 μM for the K_m and 3.76 nmol/min per mg for the V_{max}, are similar to the K_m of 0.20 μM and the V_{max} of 5.3 nmol/min per mg previously reported for BLMV isolated using this procedure [7].

Discussion

These studies demonstrate that there is a marked decrease in the capacity of adult BLMV to transport Ca^{2+} compared to that of young BLMV. The kinetic studies showed that the decrease was due to a 5-fold decrease in V_{max} with no change in the apparent affinity (Fig. 3). These findings suggest that there is a decreased number, or a decreased turnover rate, of Ca^{2+} transporters in BLMV from adult rats compared to that from young rats. However, the Ca^{2+} transporters in the adult are fully functional in terms of affinity for Ca^{2+}.

ATP-dependent Ca^{2+} uptake by BLMV correlated well with Ca^{2+} transport by the intact tissue in terms of age and intestinal segment (Table III). In the proximal duodenum, there is a marked decrease in the rate of BLMV Ca^{2+} uptake, tissue Ca^{2+} uptake, and tissue active transport with age. In the young ileum, BLMV Ca^{2+} uptake and tissue uptake are both decreased compared to the young duodenum. In terms of age changes in the ileum, BLMV Ca^{2+} uptake decreases by about half with age, but tissue uptake does not change significantly. This may reflect a greater sensitivity of the BLMV procedure for measuring changes in Ca^{2+} transport with age.

Since Ca^{2+} absorption by the intestine is a multi-step process, other components in addition

to the basal lateral membrane may contribute to the decrease in Ca^{2+} absorption with age. In general, Ca^{2+} absorption is thought to involve movement of Ca^{2+} across the brush-border membrane, translocation of Ca^{2+} across the cell cytoplasm, and active translocation of Ca^{2+} across the basal lateral membrane [15]. Translocation of Ca^{2+} across the cytoplasm may involve the vitamin D-dependent Ca-binding protein, a small, soluble protein with a high affinity for Ca^{2+}.

In studies of brush-border membrane vesicles, no changes in Ca^{2+} uptake with age were seen in F344 rats aged 2–3, 12–14 and 22–24 months [16]. The same brush-border membrane vesicle preparations show marked age-related changes in glucose uptake [12]. However, it should be noted that under the experimental conditions employed, Ca^{2+} uptake by brush-border membrane vesicles is insensitive to osmotic lysis and represents almost exclusively Ca^{2+} binding to the plasma membrane. This is in contrast to Ca^{2+} uptake by BLMV (Table II). Ca^{2+} binding to the brush-border membrane may mask possible age-related changes in Ca^{2+} uptake across the brush-border membrane.

In terms of Ca-binding protein, there is a marked decrease in intestinal Ca-binding protein with age, and this decrease parallels the decrease in the active transport of Ca^{2+} [2]. It is also of interest that this protein has been reported to stimulate the Ca^{2+}-ATPase activity of erythrocyte membranes [17]. Thus, a decreased amount of intestinal Ca-binding protein could result in decreased Ca^{2+} absorption in two ways. First, decreased Ca-binding protein could result in decreased movement of Ca^{2+} across the cytoplasm to the basal lateral membrane pump. Second, decreased Ca-binding protein could result in a decreased Ca^{2+}-ATPase activity and decreased Ca^{2+} pumping capacity of the BLMV, if this protein does indeed regulate the BLMV pump.

The mechanism responsible for the decline in pumping capacity by BLMV with age requires further investigation. Since the pumping capacity of BLMV is stimulated by 1,25-dihydroxyvitamin D [8], the decline may reflect the marked decrease in serum 1,25-dihydroxyvitamin D in rats between 2 and 12 months [16]. Vitamin D deficiency has been shown to decrease the V_{max} of Ca^{2+} uptake without altering the K_m [8]. Decreased pump capacity may also reflect an age-related alteration in the BLMV Ca-ATPase, although the role of the Ca-ATPase in Ca^{2+} pumping has been questioned [18]. Alternatively, the decreased pump capacity with age may reflect a lack of some co-factor, such as Ca-binding protein [17] or calmodulin [4,6], which is necessary for optimal activity. Finally, altered pumping capacity may reflect age-related changes in the composition and structure of the basal lateral membrane. Such changes have been described for intestinal brush-border membranes [19].

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References

1 Alevizaki, C.C. Ikkos, D.G. and Singhelakis, P. (1973) J. Nucl. Med. 14, 760–762.
2 Armbrecht, H.J., Zenser, T.V., Bruns, M.E.H. and Davis, B.B. (1979) Am. J. Physiol. 236, E769–E774.
3 Armbrecht, H.J. (1986) Biochim. Biophys. Acta 882, 281–286.
4 Nellans, H.N. (1981) J. Biol. Chem. 256, 9932–9936.
5 Hildman, B., Schmidt, A. and Murer, H. (1982) J. Membr. Biol. 65, 55–62.
6 Ghijsen, W.E.J.M., Van Os, C.H., Heizmann, C.W. and Murer, H. (1986) Am. J. Physiol. 251, G223–G229.
7 Ghijsen, W.E.J.M., De Jong, M.D. and Van Os, C.H. (1982) Biochim. Biophys. Acta 689, 327–336.
8 Ghijsen, W.E.J.M. and Van Os, C.H. (1982) Biochim. Biophys. Acta 689, 170–172.
9 Stern, B.K. (1966) Gastroenterology 51, 855–867.
10 Reinila, M., MacDonald, E., Salem, N., Linnoila, M. and Trams, E.G. (1982) Anal. Biochem. 124, 19–26.
11 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
12 Doubek, W.G. and Armbrecht, H.J. (1987) Mech. Ageing Dev. 39, 91–102.
13 Pershadsingh, H.A. and McDonald, J.M. (1980) J. Biol. Chem. 255, 4087–4093.
14 Dixon, W.J. and Massey, F.J. (1969) Introduction to Statistical Analysis, McGraw, New York.
15 Wasserman, R.H., Armbrecht, H.J., Shimura, F., Meyer, S. and Chandler, J.S. (1984) Prog. Clin. Biol. Res. 168, 307–312.

16 Armbrecht, H.J. (1988) In Aging in Liver and Gastrointestinal Tract (Bianchi, L., Holt, P.R., James, O.F.W. and Butler, R.N., eds.), pp. 131–139, MTP Press, Lancaster.
17 Morgan, D.W., Welton, A.F., Heick, A.E. and Christakos, S. (1986) Biochem. Biophys. Res. Commun. 138, 547–553.
18 Moy, T.C. Walters, J.R.F. and Weiser, M.M. (1986) Biochem. Biophys. Res. Commun. 141, 979–985.
19 Brasitus, T.A., Yeh, K.Y., Holt, P.R. and Schachter, D. (1984) Biochim. Biophys. Acta 778, 341–348.