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Sulfonates are low-affinity ligands for the GDP-binding site of brown-fat mitochondria

Jan Nedergaard *, Barbara Cannon

The Wenner-Gren Institute, The Arrhenius Laboratories F3, Stockholm University, S-106 91 Stockholm, Sweden

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Abstract

In order to study the function of the brown-fat specific uncoupling protein thermogenin (UCP), the effect of certain sulfonates on [^{3}H]GDP binding to the GDP-binding site of brown adipose tissue mitochondria was studied. The affinity of [^{3}H]GDP for the site was 1.3 μM in the normal sucrose medium, but the apparent K_D was increased to $\approx$ 20 μM in 100 mM hexanesulfonate medium. This increase in apparent K_D was found to be due to a competitive binding of hexanesulfonate to the GDP-binding site; the affinity of hexanesulfonate was only 13 mM but this was sufficient to affect the apparent affinity of GDP under experimental conditions. Also in KCl-medium, the affinity of GDP was high ($\approx$ 3 μM), but both in a benzenesulfonate medium and in a *para*-aminobenzenesulfonate (sulfanilate) medium, the apparent affinity was lower ($\approx$ 12 μM); as benzenesulfonate is well transported by thermogenin but sulfanilate is not, the reduction in affinity was unrelated to transport. In agreement with earlier data (Jezek, P. and Garlid, K.D. (1990) J. Biol. Chem. 265, 19303–19311), the potency of GDP to inhibit transport was dependent on the species transported; the fact that GDP potency was lower for benzenesulfonate transport (EC_{50} = 324 μM) than for Cl^- transport (EC_{50} = 32 μM) could adequately be explained by the competitive interaction of benzenesulfonate with the GDP-binding site, but this effect could only partly explain the even lower potency of GDP to inhibit hexanesulfonate transport (EC_{50} = 4074 μM). It was concluded that these types of substrate for thermogenin-mediated transport may directly interact with the GDP-binding site, but that this effect could only partly explain the dependence of GDP potency on substrate species.

Key words: Thermogenin; Sulfonate; GDP binding; Purine nucleotide; Mitochondrion; Brown fat; (Hamster)

1. Introduction

Despite great progress in the understanding of the molecular biology of the uncoupling protein thermogenin, the mechanism of action of this brown-fat specific mitochondrial membrane protein is still far from clarified [1–3].

The physiological activity of thermogenin is supposed to be the transmembrane transport of H^+ (equivalents) over the mitochondrial membrane, but besides being a functional protonophore, thermogenin is also able to transport certain anions. It has generally been assumed that only a few (inorganic) anions, such as Cl^-, were substrates for thermogenin-mediated transport, but Jezek and Garlid [4,5] have observed that a wide range of anions are transported by thermogenin, many of these at rates that are higher than that of the classical transported species Cl^-. It is characteristic for thermogenin that its transport activity is inhibitable by GDP (and other purine nucleotides). That the transport of the 'new' substrates was really mediated by thermogenin was supported by the fact that also their permeation was inhibited by GDP. However, Jezek and Garlid [4] also observed that the potency of GDP to inhibit transport was lower when the new, rapidly transported substrates were being transported than when, for example, Cl^- was transported. Present models for thermogenin function generally assume that GDP inhibits transport by binding to a site on thermogenin and that this is an allosteric, regulatory site. Clearly, such models would predict that the nature of the substrate and the rate of its transport would not influence the potency of the inhibitor.

* Corresponding author. Fax: +46 8 156756.

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In order to understand the dependence of GDP potency on the transported substrate, several suggestions can be formulated. One possibility would be that the affinity of GDP for its binding site is unaffected by the presence of the substrates, but that the new substrates would influence the protein in such a way that the inhibition would no longer be transduced to the catalytic part of the molecule; in other words, these compounds could be a family of true 'decouplers', examples of which have been searched for experimentally but not so far identified. Another possibility would be that the presence of the new substrates in the experimental medium would diminish the apparent K_D of GDP for binding to its site, and that it would be this decreased affinity which would lead to the diminished potency of GDP to inhibit thermogenin activity. Such an interaction could be either competitive (i.e., the new substrates could be ligands for the site) or noncompetitive (they could in other ways interfere with the physical characteristics of the site). Such interactions are not unlikely, as the substrates are present at rather high concentrations (50–100 mM) during transport experiments, and they could therefore, despite a comparatively low affinity, still be able to affect the apparent characteristics of the binding site. Indeed, Cl^- ions, with very low affinity ($K_i \approx 340$ mM) and pyrophosphate ions, with a somewhat higher affinity ($K_i \approx 25$ mM) have been demonstrated to inhibit [^{3}H]GDP binding to brown-fat mitochondria [6]. It is therefore not unlikely that other anions, such as the new substrates, could display a higher affinity for the GDP-binding site than Cl^-, and that this property could explain the lower potency of GDP to inhibit transport of the new substrates than of Cl^-.

We have therefore investigated whether the new substrates are able to influence the apparent affinity of GDP for its binding site. We conclude that the organic anions investigated are low-affinity ligands for the GDP-binding site, and that this can partially explain the decreased potency of GDP. However, for the best transported substrates, it would seem that a further mechanism must still be postulated, as the decrease in potency of GDP to inhibit transport is greater than the decrease in apparent affinity of GDP for its site.

2. Materials and methods

Brown-fat mitochondria

Brown-fat mitochondria were prepared, principally as earlier described [7], from adult Syrian hamsters which had been acclimated (one hamster per cage) to 4°C for at least 4 weeks. Routinely, one hamster was used per preparation. The hamster was killed by decapitation, and the periaortic, interscapular, cervical and axillary brown adipose tissue dissected out and combined. The tissue was minced with scissors and homogenized in about 40 ml 250 mM sucrose solution, filtered through gauze and centrifuged (all centrifugations for 10 min) at $8500 \times g$. The supernatant was discarded and the pellet resuspended in about 40 ml sucrose solution and centrifuged at $800 \times g$. The pellet was discarded and the supernatant centrifuged at $8500 \times g$. The resulting mitochondrial pellet was resuspended in 5 ml sucrose solution with 2% fatty-acid-free bovine serum albumin (Boehringer-Mannheim) [8] and centrifuged at $8500 \times g$. The pellet was resuspended in 5 ml sucrose solution and centrifuged at $8500 \times g$. The protein concentration in the resulting, albumin-washed mitochondrial pellet was measured with the fluorescamine method with Fluram (Fluka). The suspension was diluted with sucrose solution to a stock concentration of 10 or 20 mg per ml.

[^{3}H]GDP-binding capacity

The amount of [^{3}H]GDP binding was determined principally as earlier described [9], following the method of Nicholls [10]. The mitochondria (0.5 mg protein) were incubated at room temperature for 10 min in 0.5 ml of a medium which always contained 25 mM sucrose (from the mitochondrial suspension), 20 mM Tes, 1 mM EDTA, 5 μM rotenone, 0.05 μM [^{3}H]GDP (Amersham, 10 Ci/mmol, 350 000 cpm/ml), GDP (sodium salt, Sigma) added to the indicated concentrations, and 0.25 mM [^{14}C]sucrose (Amersham, 0.6 Ci/mmol, 300000 cpm/ml). The main constituent was either 100 mM sucrose (BDH), or 100 mM hexanesulfonate (sodium salt, Sigma), or 100 mM benzenesulfonate (sodium salt, Sigma) or 100 mM sulfanilate (4-aminobenzenesulfonic acid, Schering). All media were pH-adjusted to 7.1 with NaOH. After the 10 min incubation, 400 μl of the incubation was filtered through a Sartorius 0.4 μm filter. The filter was dried, dissolved in 5 ml Scintillation 299 (Packard) and counted in a liquid scintillation spectrometer.

Mitochondrial anion permability

The mitochondrial anion permeability was estimated from measurements of the initial swelling rate. Brown-fat mitochondria (0.2 mg protein) were added to a cuvette containing 1 ml of a medium which always contained 20 mM Tes, 3 mM EDTA, 5 μM rotenone, 2.5 mM sucrose (from the mitochondrial suspension), and 1 μg oligomycin. The main constituent was either 100 mM KCl + 0.5 μM valinomycin, or 100 mM sodium hexanesulfonate, sodium sulfanilate or sodium benzenesulfonate, each of these together with 0.3 μM FCCP and 1 μM monensin. All media were pH-adjusted to 7.2 with KOH or NaOH. The initial swelling rate was followed at 23°C in an Aminco dual wavelength spectrophotometer as the decrease in absorbance at 520 nm after the addition of mitochondria.

Data analysis

The experimental data were analysed with the reiterative general curve-fitting function of the KaleidaGraph data analysis/graphics application for the Macintosh computer, for adherence to the equations stated in the legends to figures.

3. Results

Jezek and Garlid [4] demonstrated that a large number of compounds, belonging to different chemical classes, were transported by thermogenin. We have here concentrated on a few members of the sulfonate class of substrates. We have chosen to investigate hexanesulfonate which was reported to be the alkylsulfonate which had the highest transport rate without showing any tendency to detergent activity. Further, we have investigated the analogues benzenesulfonate and sulfanilate which, although being chemically different by only one amino group, show contrasting behaviour as substrates for anion transport in brown-fat mitochondria (benzenesulfonate is well transported but sulfanilate not at all). We have investigated whether any of these compounds had any effect on the apparent K_D for [^{3}H]GDP binding to brown-fat mitochondria, and whether such effects were of sufficient magnitude to affect the potency of GDP to inhibit anion transport.

Effect of hexanesulfonate on [^{3}H]GDP binding characteristics

In Fig. 1, results from equilibrium binding experiments with [^{3}H]GDP are shown. In the sucrose medium routinely used for such determinations, a K_D value for [^{3}H]GDP binding to brown-fat mitochondria of 1.3 ± 0.4 μM was obtained, and the total number of [^{3}H]GDP-binding sites was found to be 0.85 ± 0.08 nmol per mg mitochondrial protein. These values are similar to those earlier obtained by different groups in these animals [9–12].

When the binding experiments were performed in a medium in which 10 mM of the sucrose was exchanged for hexanesulfonate, a change in the [^{3}H]GDP-binding characteristics was observed: the K_D value was increased to 2.2 ± 0.2 μM but the total number of binding sites was practically unchanged (0.92 ± 0.04 nmol). When [^{3}H]GDP binding was assessed in 100 mM hexanesulfonate, it became difficult to ascertain the binding characteristics with precision, but by the reiterative curve fitting method a K_D of 19 ± 8 μM was estimated. The total number of binding sites was still estimated to be unchanged (0.95 ± 0.28 pmol).

It was concluded from this initial experimental series that hexanesulfonate had significant effects on the apparent affinity of [^{3}H]GDP for its binding site, but

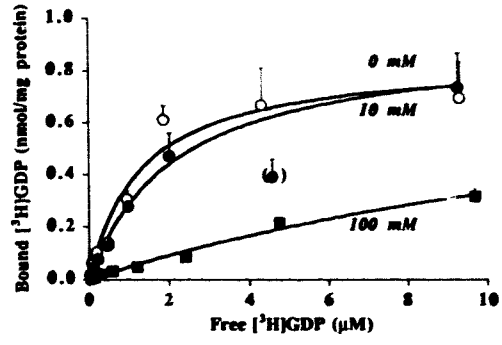

Fig. 1. Equilibrium binding curves for [^{3}H]GDP binding to brown-fat mitochondria in the presence of hexanesulfonate. [^{3}H]GDP binding to brown-fat mitochondria was performed as described in Materials and Methods. Results shown are the means + S.E. from five determinations on as many different mitochondrial preparations (where not visible, the S.E. was smaller than the size of the symbol), in buffers in which sucrose was exchanged with the indicated concentrations of hexanesulfonate. The values for free [^{3}H]GDP were estimated as the nominal (added) values minus the mean amount of bound [^{3}H]GDP. The data were analysed and the lines drawn by reiterative fitting of the means to a simple Michaelis-Menten-type equation ($B_F = B_{max} \cdot (F/(K_D + F))$; the resulting values are given in the text. The indicated outlier was not included in the calculation. Pearson's correlation coefficients were higher than 0.98.

that the total number of binding sites was unaffected by the presence of hexanesulfonate. This would indicate that hexanesulfonate was a competitive inhibitor of GDP binding.

Estimation of the K_i for hexanesulfonate

To obtain a K_i value for hexanesulfonate as a competitive ligand for the GDP-binding site on brown-fat mitochondria, the mitochondria were incubated with a fixed low amount of [^{3}H]GDP (1.5 μM, i.e., close to the K_D), in the presence of different concentrations of hexanesulfonate.

As seen in Fig. 2, the amount of [^{3}H]GDP bound was decreased as an effect of increased hexanesulfonate addition. When the data points were analysed for adherence to a competitive interaction of hexanesulfonate and [^{3}H]GDP on one binding site, the K_i for hexanesulfonate for the binding site was calculated to be 13 ± 3 mM. This estimate of the affinity was in reasonable agreement with the results shown in Fig. 1, as the apparent affinity of [^{3}H]GDP would be expected to be increased by a factor of $(1 + 10/13 =)$ 1.8 in 10 mM hexanesulfonate (the observed increase was $2.2/1.3 = 1.7$), and by a factor of $(1 + 100/13 =)$ 9 in 100 mM hexanesulfonate (the observed increase was $19/1.3 = 15$).

Based on these data, it would seem that hexanesulfonate is a ligand for the so-called GDP-binding site on brown-fat mitochondria, with a very low affinity compared to that of GDP, but still with an affinity suffi-

ciently high that it could affect the apparent affinity of GDP for this binding site under experimental conditions. Through this competitive interaction with the binding of GDP, the presence of hexanesulfonate could at least partially explain the reported lower potency of GDP for inhibition of hexanesulfonate transport than for inhibition of Cl^- transport.

[^{3}H]GDP-Binding experiments are normally performed, as here, in sucrose medium, but studies of Cl^- transport are necessarily performed in an ionic medium, normally KCl. To be able to directly compare the affinity of the GDP-binding site for GDP under conditions which resemble those in transport experiments, we performed [^{3}H]GDP-binding experiments in 100 mM KCl and in 100 mM Na^+-hexanesulfonate media. In consequence of the observed decrease in apparent GDP affinity in a hexanesulfonate medium, we performed these [^{3}H]GDP-binding experiments with a range of [^{3}H]GDP concentrations which would allow for an approach to binding-site saturation, if the interaction was competitive.

As seen in Fig. 3, and in agreement with earlier studies [13], also in KCl medium the affinity of [^{3}H]GDP for its binding site was high, with a binding constant of 2.6 ± 0.4 μM, i.e., only slightly higher than that observed above in the sucrose medium (1.3 μM); the number of binding sites was also as expected (0.66 $\pm$ 0.01 nmol per mg mitochondrial protein). In the hexanesulfonate medium, the apparent affinity of [^{3}H]GDP was increased to 23 ± 6 μM, in agreement with data above, and the estimated total number of binding sites was as expected (0.76 ± 0.08 nmol/mg protein). It should be noted that the curve shape clearly indicated that the interaction between hexanesulfonate and [^{3}H]GDP was competitive in nature, as the binding curves approached each other at high [^{3}H]GDP concentrations.

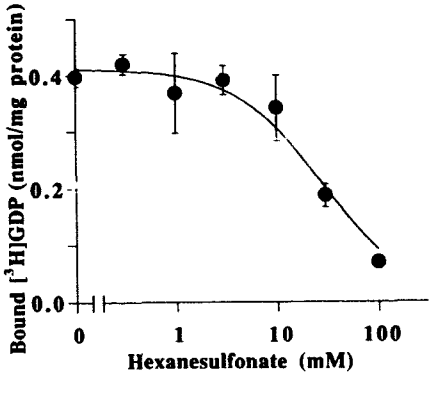

Fig. 2. Effect of increasing hexanesulfonate concentration on [^{3}H]GDP binding to brown-fat mitochondria. Brown-fat mitochondria were incubated in the presence of 1.5 μM [^{3}H]GDP, in a medium in which the indicated concentrations of sucrose were exchanged for hexanesulfonate. The values are means $\pm$ S.E. from three determinations, performed on as many mitochondrial preparations. The data were analysed and the lines drawn by reiterative fitting of the means to the equation for competitive binding, i.e., $B_I = B_{max} \cdot L/(K_D \cdot (1 + I/K_i) + L)$ where L is the concentration of [^{3}H]GDP (here 1.5 μM), K_D is the affinity of [^{3}H]GDP (here a value of 1.3 μM, obtained from Fig. 1, was used), I is the concentration of hexanesulfonate present and K_i is the inhibitory constant for hexanesulfonate on [^{3}H]GDP binding. The computer fitting yielded a total number of sites (B_{max}) of 0.77 ± 0.03 nmol/mg, a value close to that obtained in Fig. 1. The K_i for hexanesulfonate was calculated to be 13 ± 3 mM. Pearson's correlation coefficient was 0.98.

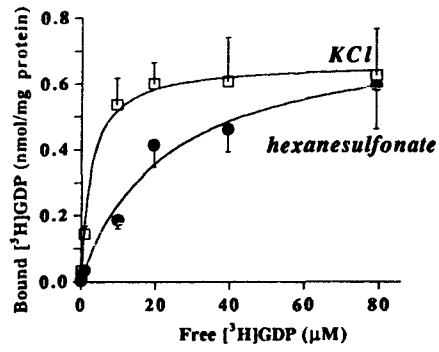

Fig. 3. Equilibrium binding curves for [^{3}H]GDP binding to brown-fat mitochondria in hexanesulfonate or KCl media. Brown-fat mitochondria were incubated as described in Fig. 1, except that the incubation medium consisted of 100 mM sodium hexanesulfonate or 100 mM KCl instead of sucrose. Results are means and S.E. from 5 experiments, performed on as many mitochondrial preparations. Calculations were performed as described in the legend to Fig. 1. Pearson's correlation coefficients were 0.99 in both media.

A comparison of the effect of benzenesulfonate and sulfanilate

It was pointed out by Jezek and Garlid [4] that the two chemical analogues benzenesulfonate and sulfanilate (the *para*-amino analogue of benzenesulfonate) show very different transport characteristics in brown-fat mitochondria: benzenesulfonate is well transported (similar to Cl^-) whereas sulfanilate is not transported at all, nor does it inhibit Cl^- transport. We therefore wanted to investigate whether this contrasting behaviour in transport ability was also reflected in an effect on apparent [^{3}H]GDP affinity, i.e., whether the property of being a substrate for transport was associated with an effect on the characteristics of the GDP-binding site.

As seen in Fig. 4, this was not the case: the two compounds had practically identical effects on the apparent parameters for [^{3}H]GDP binding. The apparent K_D increased from 0.8 ± 0.2 μM in sucrose medium to approx. 12 μM in both benzensulfonate analogues (11.7 ± 1.4 in benzensulfonate and 12.4 ± 2.2 in sulfanilate). The estimated total number of binding sites was similar in the sucrose and the sulfanilate media (0.58 ± 0.02 nmol per mg protein versus 0.52 ± 0.03) but this

number was somewhat higher in benzenesulfonate (0.70 ± 0.03). Thus, the principal difference in the transport ability of the two substances was not reflected in any difference in their effect on the apparent characteristics of the GDP-binding site.

The potency of GDP as a transport inhibitor

From the data above, it is clear that the sulfonates were able to interact competitively with the GDP-binding site and thus, qualitatively, possessed the property which could explain the lower potency of GDP to inhibit the transport of these substrates. In order to investigate whether the quantitative effects were of a magnitude sufficient to explain the decreased potency of GDP, we examined the ability of GDP to inhibit anion transport in brown-fat mitochondria, – i.e., we repeated the original observations of Jezek and Garlid but under conditions similar to those used by us for the [^{3}H]GDP-binding studies reported above. The parameter used here for transport rate was the initial tangent to the decrease in absorbance observed during mitochondrial swelling under conditions where anion permeability is the rate-limiting factor. This parameter may not be fully proportional to the actual transport rate, but as we were primarily concerned with changes in the EC_{50} value for GDP, possible deviations from linearity should only marginally influence the results.

In agreement with the results of Jezek and Garlid, it is evident from the results depicted in Fig. 5 that the EC_{50} for GDP as an inhibitor of anion transport depended on the ion species transported. For the classical transport of Cl^-, the apparent EC_{50} for GDP as an inhibitor was 32 ± 9 μM, a value close to values earlier observed with this method in our laboratory [7], but

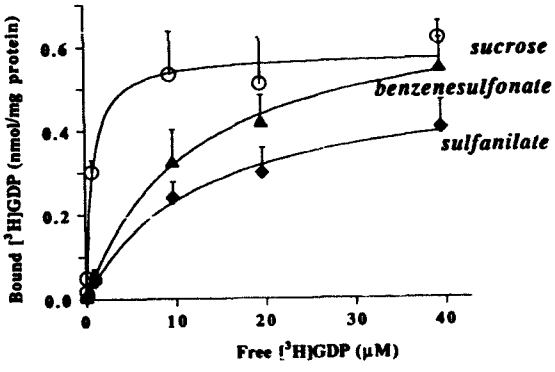

Fig. 4. Equilibrium binding curves for [^{3}H]GDP binding to brown-fat mitochondria in the presence of sucrose, benzenesulfonate and sulfanilate. Brown-fat mitochondria were incubated as described in Fig. 1, except that the incubation medium, where indicated, consisted of 100 mM benzenesulfonate or 100 mM sulfanilate. Results are means + S.E. from three experiments, performed on as many mitochondrial preparations. The results were analysed as described in the legend to Fig. 1. Pearson's correlation coefficients were 0.99 in all cases.

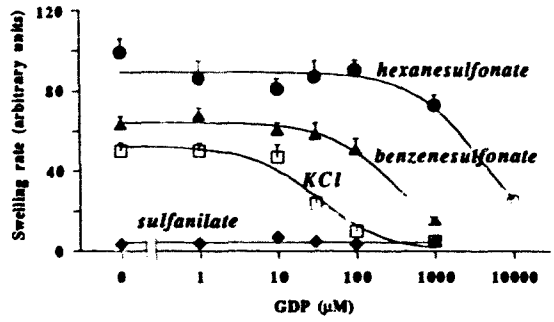

Fig. 5. Inhibitory effect of GDP on passive swelling in different media. Brown-fat mitochondria were added to cuvettes containing the indicated media, and the initial rate of swelling was measured as described in Methods. Results are means + S.E. from four experiments, performed on as many mitochondrial preparations. The mean data points were analysed by reiterative fitting to a Michalis-Menten type kinetics $V_G = V_{max} - V_{max} \cdot (G/(EC_{50} + G))$ where G is the GDP concentration. No meaningful analysis could be made of the sulfanilate data (the arbitrary swelling rate was ≈ 4). The resulting values for V_{max} and EC_{50} are presented in the text; Pearson's correlation coefficients for KCl, hexanesulfonate and benzenesulfonate were 0.98, 0.99 and 0.97, respectively.

somewhat higher than that reported by others [14,15]. However, as the present experiments were performed at pH 7.2 and those in [14, 15] at pH 7.0, this difference is that expected from the known high pH-dependence of the interaction of GDP with the binding site [3].

Also in our hands, benzenesulfonate was transported well by thermogenin in brown-fat mitochondria. The transport rate for benzenesulfonate observed here was somewhat (23%) higher than that of the classical substrate Cl^- (Jezek and Garlid reported a 20% lower rate). The apparent EC_{50} of GDP for inhibition of benzenesulfonate transport was much higher than that for inhibition of Cl^- transport: 324 ± 49 μM versus 32 ± 9 mM. This shift in EC_{50} value can be compared with that observed above for the effect of benzenesulfonate on apparent [^{3}H]GDP affinity: the K_D for [^{3}H]GDP was ≈ 2.6 μM in KCl medium, and ≈ 12 μM in benzenesulfonate medium, i.e., an approx. 5-fold shift in the same direction. Thus, for this anion, most – if not all – of the lower potency of GDP to inhibit the transport of benzenesulfonate than to inhibit the transport of Cl^- could be explained by the fact that benzenesulfonate competitively inhibited the binding of GDP to its regulatory site.

Also in agreement with the results of Jezek and Garlid, sulfanilate was not a substrate for transport in the mitochondria (Fig. 5); thus no comparison of the relation between the K_D and the EC_{50} could be made; it should, however, be remembered that the apparent K_D was markedly decreased despite the fact that sulfanilate was not a substrate for transport. Since sulfani-

late was also unable to inhibit Cl^- transport [4], the decreased GDP affinity in the presence of sulfanilate could hardly be due to the formation of a nontransported intermediate of sulfanilate-thermogenin.

In the case of hexanesulfonate (Fig. 5), the situation was more complex. The maximal transport rate estimated with the swelling method was higher than that of the classical substrate Cl^-, as well as that of benzenesulfonate, but the rate was only 71% higher than that of Cl^-, whereas Jezek and Garlid observed a 424% higher rate. In agreement with Jezek and Garlid, we found that the potency of GDP to inhibit transport was low (as compared to that seen with Cl^- as the transported species); the EC_{50} value was no less than 4074 ± 1138 μM, i.e., 127-fold higher than that for inhibition of Cl^- transport. This shift was higher than that observed by Jezek and Garlid, who found a 35-fold increase with 54 mM hexanesulfonate [5]). The shift was also higher than that which could be explained by the decreased apparent affinity of GDP for the binding site. According to the data above, the affinity decreased from ≈ 2.6 μM in the KCl medium to ≈ 23 μM in the hexanesulfonate medium, i.e., only a 9-fold shift in affinity. Thus, inhibition of hexanesulfonate transport requires two orders of magnitude more GDP than inhibition of Cl^- transport. Of this reduction in GDP potency, only one order of magnitude could be explained by hexanesulfonate competition for the GDP-binding site; another mechanism must be responsible for the further $\approx$ 10-fold decrease in potency.

4. Discussion

In the present study, we have investigated whether the 'new' anionic substrates for thermogenin-mediated transport over the inner mitochondrial membrane of brown-fat mitochondria influence the apparent affinity of GDP for its binding site on the mitochondria. We found that the compounds investigated here, which all are sulfonates, are all ligands for the GDP-binding site. Their affinity is very low, in the 10 mM range, i.e., about 10000 fold lower than that of GDP for the site. Nevertheless, as these compounds are present in high mM concentrations when their transport is being investigated, they must influence the apparent affinity of GDP for the binding site and in this way increase the EC_{50} of GDP about 10-fold. For certain transported species, this decrease in apparent GDP affinity, due to the competition, could seemingly fully explain the increased EC_{50}, but for other transported species, this effect could not explain the increase in EC_{50} observed, and other explanations must be found.

In the classical model of thermogenin function, the GDP-binding site is considered a regulatory, allosteric site, and binding of an agonist (such as GDP) to this site results in an inhibition of Cl^- transport. In such a model, it would be expected that the K_D for GDP binding to the site would be identical to the EC_{50} for GDP inhibition of anion transport. A limitation of this model is the observed difference between the affinity of agonists (GDP) for the binding site and their EC_{50} as inhibitors of thermogenin activity (transport). Concerning Cl^- transport, such a discrepancy has long been recognized [7]. Also in the present study, we observe for all transported substrates an apparent affinity of GDP for its binding site which is higher than its potency as an inhibitor of transport in the same medium: 10-fold higher for Cl^-, 30-fold higher for benzenesulfonate and 200-fold higher for hexanesulfonate. In the present study, we have demonstrated that this increased discrepancy can only partly be explained by the competitive action of the different new substrates on the GDP-binding site, implying that another mechanism must exist. It may be noted that the discrepancy between the K_D and the EC_{50} values is higher when better transported species are investigated. Principally, this would lend support to the idea of Rial and Nicholls [1] that a competition between the anion-transporting and the GDP-binding forms of thermogenin exists, so that more easily transported substrates would decrease the apparent affinity for GDP. However, it has been mentioned by Jezek and Garlid [4] that the system does not exhibit the expected symmetrical behaviour (high GDP does not decrease anion affinity), so that this model also has limitations in explaining the existing data and the data presented here concerning the relationship between the K_D and the EC_{50} for GDP interaction with the uncoupling protein thermogenin.

Acknowledgements

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